DISCOVERY OF MAMMALIAN D-CYSTEINE

by
Moataz M. Gadalla

A dissertation submitted to Johns Hopkins University in conformity with the requirements for the degree of Doctor of Philosophy

Baltimore, Maryland

May, 2017

ABSTRACT

Among the amino acids with the fastest *in vitro* spontaneous racemization rates, the D-stereoisomer of aspartic acid and serine are found endogenously in mammalian systems. However, the D-stereoisomer of the amino acid with the fastest *in vitro* spontaneous racemization rate, cysteine, has never been examined. We use high-performance liquid chromatography utilizing a chiral crown ether column as a chiral selector to establish the presence of endogenous mammalian D-cysteine. Based on the fact that D-cysteine is used in the chemical synthesis of D-luciferin, the substrate for firefly luciferase, from 2-cyano-6-hydroxybenzothiazole, a luciferase bioluminescence assay for the stereo-specific detection of D-cysteine is used. The assay readily detects 0.1-1.0 μM D-cysteine and so is suitable for monitoring physiologic mammalian tissue levels of D-cysteine. Based on this assay we develop a method for the *in vivo* optical imaging of endogenous D-cysteine in transgenic mice overexpressing firefly luciferase. Using this method, we find relatively high levels of D-cysteine in the eyes and a transverse thoraco-abdominal organ, which on dissection is identified as the pancreas. We identify serine racemase and D-amino acid oxidase as candidate enzymes for the endogenous biosynthesis and degradation of mammalian D-cysteine, respectively. We find that serine racemase knockout mice develop an age-related impairment in vision. Electroretinogram responses are attenuated in older serine racemase knockout mice compared to age-matched wildtype controls. Both scotopic a-waves and b-waves are severely attenuated, photopic b-waves are also diminished. Electroretinogram responses are unaffected in D-amino acid oxidase knockout mice compared to age-matched wildtype controls. Retinal fundus photography

indicates focal disruptions of the retinal pigmented epithelium in older serine racemase knockout mice. In toluidine blue-stained retinal sections, overall thinning of the retina is observed in older serine racemase knockout mice compared to age-matched wildtype controls.

Thesis Advisor:

Dr. Solomon H. Snyder, M.D. (Designated reader)

Thesis Committee Members:

Dr. Philip A. Cole, M.D., Ph.D.

Dr. Jun O. Liu, Ph.D.

Dr. Jonathan Pevsner, Ph.D. (Designated reader)

ACKNOWLEDGEMENTS

I would like to thank the following: My parents, siblings, wife, and son, for their unconditional love and support. My thesis advisor Dr. Solomon H. Snyder for his mentorship and support. My thesis committee members, Dr. Philip A. Cole, Dr. Jun O. Liu, and Dr. Jonathan Pevsner. The Medical Scientist Training Program administration, Sharon A. Welling, Bernadine F. Harper, and Martha A. Buntin. The Department of Pharmacology and Molecular Sciences administration, Mary [Mimi] C. Guercio and Amy Paronto. All members of the Snyder Laboratory, particularly, Urbain Weyemi, Roxanne K. Barrow, Lynda D. Hester, Lauren K. Albacarys, Adele M. Snowman, Aledia F. Carmichael, Virginia Miller, Barbara A. Zeigler, and Susan McTeer. Medical Scientist Training Program research rotation students that I mentored, Alisa Mo and Robert Wardlow. Johns Hopkins University undergraduate students I mentored in research, Tomas Rojas and Alfred Chin.

I would also like to thank my collaborators for help characterizing the eye phenotype of serine racemase knockout mice and D-amino acid oxidase knockout mice. Dr. Peter A. Campochiaro and members of his laboratory at the Johns Hopkins Wilmer Eye Institute: Dr. Yogita Kanan for electroretinograms; Sean F. Hackett for retinal fundus photography and enucleation of fixed eyes; and Mahmood Khan for electroretinograms and retinal fundus photography. Dr. Maged M. Harraz, Snyder Laboratory, for transcardial perfusions. Jeffrey D. Messinger and Dr. Christine A. Curcio, the University of Alabama at Birmingham, for serine racemase knockout mice eye histopathology.

This work was supported by the National Institutes of Health Medical Scientist Training Program Award (T32 GM007309) to Moataz M. Gadalla and U.S. Public Health Service Grant (MH018501) to Dr. Solomon H. Snyder. Portions of this thesis include contributions from my mentor Dr. Solomon H. Snyder.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1. Discovery of mammalian D-cysteine.

Summary: The presence of endogenous mammalian D-cysteine is established by high-performance liquid chromatography utilizing a chiral crown ether column as a chiral selector. A method for the *in vivo* optical imaging of endogenous D-cysteine in transgenic mice overexpressing firefly luciferase is developed. It is based on the fact that D-cysteine is used in the chemical synthesis of D-luciferin, the substrate for firefly luciferase, from 2-cyano-6-hydroxybenzothiazole. *In vivo* optical imaging of endogenous D-cysteine in transgenic mice overexpressing firefly luciferase indicate relatively high levels of D-cysteine in the eyes and a transverse thoraco-abdominal organ, which on dissection is identified as the pancreas. Candidate enzymes for the endogenous biosynthesis and degradation of mammalian D-cysteine are serine racemase and D-amino acid oxidase, respectively. Serine racemase knockout mice develop an age-related vision impairment which we characterize.

Chapter 1a. Introduction

Molecular Chirality

In 1848, Louis Pasteur first described molecular chirality studying crystals of tartrates (Pasteur, 1848a; Pasteur, 1848b; Kauffman & Myers, 1998; Gal, 2011). He explained why the newly available chemically synthesized tartrates do not rotate plane polarized light, i.e., are optically inactive, unlike naturally occurring tartrates, purified from living matter, which rotate plane polarized light, i.e., are optically active, despite otherwise identical physical properties, chemical properties, and elemental composition. He noticed that crystals of optical active naturally occurring tartrates, are hemihedral, i.e., has small facets at alternate corners, with right-handed facets. On the other hand, crystals of optically inactive chemically synthesized tartrates are not hemihedral, except for the sodium ammonium salt which has crystals that are not only hemihedral, but also enantiomorphic, i.e., some of the crystals has right-handed hemihedral facets, similar to naturally occurring tartrates, and other crystals has non-superimposable mirror image left-handed hemihedral facets. He manually separated the enantiomorphic crystal forms and found that solutions made from crystals with right-handed hemihedral facets are dextrorotatory, i.e., rotate plane polarized light clockwise to the right, while solutions made from crystals with left-handed hemihedral facets are levorotatory, i.e., rotate plane polarized light anticlockwise to the left. Solutions prepared from an equal mixture of the enantiomorphic crystal forms are optically inactive, i.e., do not rotate plane polarized light. With this, Pasteur was the first to demonstrate molecular chirality and also the first to separate the two enantiomer forms of a chiral substance. Furthermore, since naturally

occurring tartrates, purified from living matter, are enantiopure dextrorotatory, i.e., homochiral, this would be the first demonstration of homochirality in biological systems, something not completely appreciated at the time.

Biological Homochirality

Biological systems are homochiral, i.e., chiral molecules in biological systems predominantly exist as one form of the chiral enantiomers, for example, left-handed L-amino acids and right-handed D-sugars. Although the two enantiomeric forms of a chiral molecule have identical chemical and physical properties, apart from rotation of plane polarized light, the way it interacts with other chiral molecules is different, just as a right hand interacts differently with right as opposed to left hand gloves. As a result, biological homochirality is critical for molecular biological processes such as molecular recognition in substrate-enzyme and ligand-receptor binding as well as template driven processes such as DNA replication, RNA transcription, and protein translation. The origin of biological homochirality remains a matter of active study (Blackmond, 2010).

Chirality of amino acids was described by Pasteur. In 1852, he showed that aspartic acid chemically synthesized by heating ammonium fumarate is optically inactive, while aspartic acid purified from vetch plants is optically active (Pasteur, 1852). Proteins present in living organism are made exclusively from L-amino acids. This stems from the stereospecificity of the biological protein synthetic machinery (Yamane *et al.*, 1981).

In addition to being the basic building blocks of peptides and proteins, amino acids are also important metabolic precursors and intermediates in the biosynthesis of many important biological molecules such as serotonin, catecholamines, and nitric oxide,

whose amino acid precursors are L-tryptophan, L-tyrosine, and L-arginine, respectively. Furthermore, they could also function directly as biochemical messenger and signaling molecules. This includes classical amino acid neurotransmitters, such as L-glutamate and glycine; and amino acid signaling (L-leucine) in autophagy (Efeyan *et al.*, 2015).

Since L-amino acids are the predominant naturally occurring amino acids in biological systems both in proteins and as metabolic precursors and intermediates in the biosynthesis of a variety of important biological molecules, for a long time it was thought unlikely that D-amino acids were of biological significance. Although as early as 1935, Hans Krebs identified an enzyme, D-amino acid oxidase, in the liver and kidneys that oxidatively deaminates D-amino acids and not L-amino acids (Krebs, 1935). However, he was unsure of its physiological function.

Discovery of biological D-amino acids

Advancements in analytical methods for the enantiomeric separation of amino acids facilitate the discovery of D-amino acids in biological systems. Peptidoglycans in bacterial cell walls are found to contain D-amino acids, such as D-glutamate and D-alanine (Reaveley & Burge, 1972). D-amino acids are also found in insects, higher plants, and marine invertebrates (Corrigan, 1969; Robinson, 1976; D'Aniello & Giuditta, 1978; Felbeck, 1985; Felbeck & Wiley, 1987). D-amino acids in mammals are first identified in proteins that do not undergo turnover in human tooth enamel, tooth dentine, and eye lens (Helfman & Bada, 1975; Helfman & Bada, 1976; Bada, 1984). Since humans are long-lived mammals over time proteins that do not undergo turnover

accumulate D-amino acid residues by *in situ* racemization of L-amino acid residues in such proteins.

Mammalian D-aspartic acid

The first free D-amino acid to be identified in mammalian systems is D-aspartic acid (Dunlop *et al.*, 1986). They find the highest concentration of free D-aspartic acid in the cerebral hemispheres of new born rats. In adults, they find the highest concentration of free D-aspartic acid in the pituitary gland. They also observe rapidly diminishing postnatal levels of free D-aspartic acid with age, indicating that it is likely to play a rôle in development. D-aspartic acid is a poor substrate of D-amino acid oxidase (Bender & Krebs, 1950). It is degraded by the peroxisomal enzyme D-aspartate oxidase. Using FAD as a cofactor it oxidizes D-aspartate to oxaloacetate, ammonia, and hydrogen peroxide. D-aspartate oxidase knockout mice have markedly elevated tissue levels of D-aspartate (Huang *et al.*, 2006). They have markedly increased D-aspartate immunoreactivity in the adrenal cortex together with basally elevated serum corticosterone concentration compared to wildtype mice (Weil *et al.*, 2006). The intermediate lobe of the pituitary gland, endogenously express D-aspartate oxidase and as a result has negligible levels of D-aspartate. In D-aspartate oxidase knockout mice the pituitary intermediate lobe exhibits elevated levels of D-aspartate coupled with significantly reduced levels of proopiomelanocortin and α-melanocyte-stimulating hormone, a melanocortin. Consistent with diminished levels of α-melanocyte-stimulating hormone, D-amino acid oxidase knockout mice exhibit deficits in melanocortin-dependent behaviors, namely, increased weight gain, impaired sexual performance, and

decreased autogrooming (Huang *et al.*, 2006). In addition, D-aspartate oxidase knockout mice exhibit reduced immobility in the Porsolt forced swim test, and significant deficits in prepulse inhibition and rotarod performance (Weil *et al.*, 2006).

Mammalian D-serine

The second free D-amino acid to be identified in mammalian systems is D-serine (Hashimoto *et al.*, 1992a). Using gas chromatography, they succeed in separating *N*,*O*-pentafluoropropionyl isopropyl derivatives of D-serine and L-serine in rat brain extracts. The identity of the D-serine peak is confirmed using electron impact and positive chemical ionization mass spectrometry. They estimate rat brain D-serine levels to be ~0.27 mM, ~23% of the total serine in the brain. They reproduce these findings using high-performance liquid chromatography (HPLC) in which amino acids derivatized with N-*tert.*-butyloxycarbonyl-L-cysteine and *o*-phthaldialdehyde are separated using HPLC followed by fluorimetric detection (Hashimoto *et al.*, 1992b). Using HPLC, rat brain D-serine levels are determined to be ~0.22 mM, ~25% of the total serine in the brain.

Hashimoto *et al.*, 1993, find that the brain distribution of D-serine resembles that of *N*-methyl-D-aspartate (NMDA) receptors. D-serine is found to be 3-4 times more potent in activating the NMDA receptors than glycine itself (Matsui *et al.*, 1995). Degradation of D-serine by D-amino acid oxidase markedly reduced NMDA neurotransmission (Mothet *et al.*, 2000). This supports the notion that D-serine is the endogenous ligand of the 'glycine' site of the NMDA receptor (Snyder & Kim, 2000; Mustafa *et al.*, 2004). Using an antibody against glutaraldehyde conjugated D-serine, immunoreactivity of D-serine is found to correlate with the distribution of NMDA

receptors, and inversely correlate with D-amino acid oxidase (Schell *et al.*, 1995). Namely, high densities of D-serine are observed in the forebrain with extremely low densities in the hindbrain. Inversely, D-amino acid oxidase is almost non-existent in the forebrain, with high densities in the hindbrain. The reciprocal localization of D-amino acid oxidase relative to D-serine indicates that D-amino acid oxidase is the endogenous enzyme responsible for D-serine degradation. Indeed, mutant mice and rats that lack D-amino acid oxidase have markedly elevated levels of D-serine in the hindbrain which would otherwise have negligible D-serine levels (Yamanaka *et al.*, 2012). This is consistent with the observed high expression of D-amino acid oxidase in the hindbrain.

The conversion of radiolabeled L-serine to D-serine was first described in the pupae of silkworm, *Bombyx mori* (Sirinivasan *et al.*, 1965). In mammals, conversion of radiolabeled L-serine to D-serine in brains of intact rats was also demonstrated (Dunlop & Neidle, 1997), indicating that D-serine is likely formed by the direct racemization of L-serine by a serine racemase. The serine racemase activity in crude pupal lysates was found to be too unstable to enable purification of the enzyme. Addition of protease inhibitors and ammonium persulfate fractionation enabled the partial purification of a stably active preparation (Uo *et al.*, 1998). Similarly, no serine racemase activity was detectable in rat brain homogenates. However, ammonium persulfate fractionation enabled the partial purification of a stably active preparation that enabled the purification (Wolosker *et al.*, 1999a) and subsequent cloning (Wolosker *et al.*, 1999b) of serine racemase.

Binding interactions regulate serine racemase. Glutamate receptor interacting protein (GRIP) physiologically binds serine racemase, activating it, enhancing D-serine

release (Kim *et al.*, 2005). Glutamate receptor activation triggers GRIP binding to serine racemase and its activation. This interaction mediates the developmental migration of granular cells in the cerebellum, known to be enhanced by glutamate. GRIP adenoviral infection of neonatal cerebellum increases granule cell migration, which is impeded by degradation of D-serine with D-amino acid oxidase. Thus, in neuronal migration, glutamate stimulates Bergmann glia to form and release D-serine leading to activation of NMDA receptors on granule cells to augment migration. Metabotropic glutamate receptor activation of glia also regulates serine racemase (Mustafa *et al.*, 2009). Receptor activation stimulates phospholipase C (PLC) to degrade phosphatidylinositol (4,5)-bisphosphate (PIP2), relieving physiologic inhibition of serine racemase by PIP2. Serine racemase binds protein interacting with C-kinase (PICK1) via the PDZ domain of PICK1 (Fujii *et al.*, 2006). PICK1 is a susceptibility gene for schizophrenia. D-serine levels are decreased in the forebrain of neonatal PICK1 knockout mice (Hikida *et al.*, 2008). Serine racemase is also regulated by binding to the scaffolding protein disrupted-in-schizophrenia-1 (DISC1) which binds and stabilizes serine racemase (Ma *et al.*, 2013). The mutant form of DISC1, associated with psychosis, fails to bind serine racemase leading to the degradation of serine racemase via ubiquitination, and decreases in D-serine formation. Transgenic mice containing mutant DISC1 exhibit behavioral anomalies consistent with deficient NMDA neurotransmission. Serine racemase is depleted in these mice. Thus, the abnormal behavior associated with mutant DISC1 in animals and patients may reflect this mechanism that leads to depletion of D-serine and diminished NMDA neurotransmission. Mice with targeted deletion of serine racemase whose production of D-serine is profoundly decreased exhibit aberrant glutamatergic

neurotransmission and a variety of subtle behavioral abnormalities, reinforcing the importance of D-serine for physiologic NMDA transmission (Basu *et al.*, 2008). The rôle of D-serine as a pathologic as well as physiologic ligand for NMDA receptors is supported by findings that serine racemase deletion protects against NMDA receptor-associated excitotoxicity in stroke (Mustafa *et al.*, 2010).

Serine racemase can link α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and NMDA neurotransmission (Ma *et al.*, 2014). Serine racemase binds avidly to stargazin, a major accessory protein for AMPA receptors. This leads to membrane association of serine racemase and decreased catalytic activity. Serine racemase also binds with high affinity to postsynaptic density protein 95 (PSD95) mediated by the PDZ ligand consensus of TVSV at its C-terminus. The binding of serine racemase to stargazin provides a link to AMPA receptors. Application of AMPA dissociates serine racemase from stargazin, resulting in the internalization of serine racemase and derepression of serine racemase activity, increasing D-serine production and potentially activating NMDA receptors. These findings provide a means whereby AMPA transmission can influence NMDA transmission. This interesting cross-talk between the two receptors is mediated by translocation of a neurotransmitter biosynthetic enzyme, a rather novel concept.

Feedback by nitric oxide from post-synaptic cells also regulates serine racemase (Mustafa *et al.*, 2007). Thus, serine racemase is physiologically nitrosylated, inhibiting its catalytic activity. NMDA receptor activation enhances serine racemase nitrosylation by stimulating neuronal nitric oxide synthase (nNOS). By activating nNOS, NMDA transmission enhances serine racemase nitrosylation, supporting a model in which

stimulation of nitric oxide formation in NMDA-receptor containing neurons leads to retrograde movement of nitric oxide to presynaptic cells to decrease D-serine formation providing feedback inhibition of NMDA transmission.

There had been controversy as to the localization of D-serine and its biosynthetic enzyme serine racemase in neurons versus glia, with the consensus emerging that there are localizations in both compartments (Ehmsen *et al.*, 2013). L-serine disposition is determined in major part by 3-phosphoglycerate dehydrogenase, an exclusively glial enzyme responsible for the committed step in the phosphorylated pathway for L-serine formation. Experiments with transgenic mice expressing enhanced green fluorescent protein under the serine racemase promoter as well as knockout mice of serine racemase and/or 3-phosphoglycerate dehydrogenase establish that neuronal D-serine derives from glial supply of L-serine. This forms the basis of the glia-neuron serine shuttle model (Wolosker & Radzishevsky, 2013).

The Na^+-independent amino acid transporter alanine-serine-cysteine transporter 1 (Asc-1) is the primary transporter for neuronal D-serine reuptake in the mouse central nervous system (Rutter *et al.*, 2007). Asc-1 is expressed in the brain, lung, and small intestines. Immunohistochemistry in rodent brains indicate widespread neuronal immunoreactivity (Helboe *et al.*, 2003; Matsuo *et al.*, 2004). [^{3}H]D-serine uptake in Asc-1 knockout mice, compared to wildtype mice, is reduced to 34% in forebrain synaptosomes and 22% in cerebellar synaptosomes. On omission of Na^+ from the assay buffer, [^{3}H]D-serine uptake in Asc-1 knockout mice, compared to wildtype mice, is reduced to 8% in forebrain synaptosomes and 1% in cerebellar synaptosomes. Omission of Na^+ from the assay buffer is necessary to halt Na^+-dependent transport. The IC_{50} for

Na^+-independent D-serine uptake by Asc-1 is 19 μM. Homozygous Asc-1 knockout mice develop tremors and seizures (Xie *et al.*, 2005). Severe seizures result in their early postnatal death at 36-40 days of age. The NMDA receptor antagonist, MK-801, reduces both tremors and seizures in these mice. The fatal hyperexcitability of Asc-1 knockout mice is presumably due to NMDA receptor overactivation by elevated levels of extracellular D-serine.

Mammalian D-cysteine

We wondered what is chemically unique about D-serine, and D-aspartic acid compared to other D-amino acids for them to evolve into messenger molecules in mammalian systems. In speculating as to why D-serine and D-aspartate but not other D-amino acids exist in mammalian systems, we presumed that, thermodynamically speaking, they might be more prone to spontaneous racemization and, in the course of evolution, racemase enzymes developed to carry out the process physiologically. We started by looking up the *in vitro* spontaneous racemization rates of different amino acids (Man & Bada, 1987) [Table 1]. Interestingly, the *in vitro* spontaneous racemization half-lives of amino acids vary greatly [Table 1]. Among the amino acids with the fastest spontaneous racemization rates are serine displaying a half-life of 3 days, and aspartate displaying a half-life of 30 days [Table 1]. However, they are not the fastest; notably, the half-life for cysteine is only 2 days, the shortest of any amino acid, implying it has a greater propensity for racemization than all other physiologic amino acids [Table 1].

Interest in serine and aspartate as amino acids whose D-isomers occur physiologically in mammalian systems stemmed from screens of mammalian tissues for

D and L-isomers of all amino acids (Hashimoto *et al.*, 1992b). We wondered if in such previous studies that systematically looked at the concentration of different D-amino acid enantiomers in mammalian systems attempted but failed to demonstrate the presence of D-cysteine. To my surprise, those studies never examined cysteine whose detection would have been precluded due to interference with the derivatization procedure employed, which relied on thiol containing reagents (Hashimoto *et al.*, 1992b). The method of amino acid derivatization with N-*tert.*-butyloxycarbonyl-L-cysteine and *o*-phthaldialdehyde used in such studies (Hashimoto *et al.*, 1992b) to detect D-amino acid enantiomers using HPLC, would be unable to detect any molecule with a free thiol, such as cysteine.

Chapter 1b. Results

High-performance liquid chromatography detection of endogenous mammalian D-cysteine

Various biological thiols are fluorescently labeled with 4-(aminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (ABD-F) (Toyo'oka & Imai, 1984) [Figure 1]. Following labeling, the ABD-thiol adducts are chirally separated by direct isocratic elution of an HPLC column with a chiral crown ether, CROWNPAK® CR (+) (Chiral Technologies Inc., Wester Chester, Pennsylvania) [Figure 2]. Chiral recognition is achieved using an aqueous acidic mobile phase; pH 1.0 aqueous perchloric acid. Under such acidic conditions the amino group is protonated to form an ammonium ion which complex with the chiral crown ether inside its chiral cavity [Figure 2A]. This enables the chiral crown ether to act as a chiral selector for compounds with a primary amino group near their chiral center. The absolute configuration is inferred from the elution order. In CR (+) the D-stereoisomer elutes before the L-stereoisomer. CR (-) has reverse enantioselectivity and therefore provides the reverse order of elution relative to the CR (+), i.e., the L-stereoisomer elutes before the D-stereoisomer [Figure 2B].

The column is calibrated using known standards of commonly occurring biological thiols [Figure 3], the retention times of the standards are noted [Table 2] and used to determine the contents of tissue extracts. This enables us to discriminate and quantify D- and L-cysteine after fluorescent labeling of their thiol groups with ABD-F [Figure 4]. Utilizing this technique, when we homogenize mouse liver is in Hepes-NaOH buffer, pH 7.7, using a tissue grinder, we observe a 1:6 D:L ratio for cysteine [Figure 4D].

However, when we extract mouse liver in 1X 2 N HCl for 10 minutes at 99 ºC, we observe a 1:1 D:L ratio [Figure 4E]. We confirm that these acid extraction conditions do not catalyze the racemization of L-cysteine to D-cysteine. The fact that the D:L ratio increases following acid extraction indicates that D-cysteine might be sequestered in a cellular compartment that is not readily lysed by tissue grinder homogenization. The identity of the D-cysteine and L-cysteine peaks are confirmed by their augmentation when the samples are spiked with a racemic cysteine mixture [Figure 4F].

The luciferase bioluminescence assay for the stereo-specific detection of D-cysteine.

To evaluate the disposition of D-cysteine in tissues we use a simple, sensitive and specific assay for D-cysteine. It is based on the fact that D-cysteine is used in the chemical synthesis of D-luciferin, the substrate for firefly luciferase, from 2-cyano-6-hydroxybenzothiazole (CHBT) [Figure 5] (Niwa *et al.*, 2006; Kajiyama, 2002). In the presence of ATP, firefly luciferase metabolizes D-luciferin generating photons or luminescence. Therefore, if a mixture of CHBT, ATP, and firefly luciferase are added to a tissue extract or lysate, luminescence would only be observed if D-cysteine is present, reacting with CHBT to form D-luciferin the luminescent substrate for firefly luciferase. The assay readily detects 0.1-1.0 μM D-cysteine, and so is suitable for monitoring physiologic mammalian tissue levels of D-cysteine [Figure 6]. In the assay, using tris-(2-carboxyethyl) phosphine (TCEP) as a reducing agent provides better signal than DTT [Figure 6]. This assay forms the biochemical basis for the *in vivo* optical imaging of endogenous D-cysteine in transgenic mice overexpressing firefly luciferase.

***In vivo* optical imaging of endogenous D-cysteine in transgenic mice overexpressing firefly luciferase.**

FVB-Tg(CAG-luc,-GFP)L2G85Chco/J ($Luc^{+/+}$) mice harbor the CAG-luc-eGFP L2G85 transgene which is under the control of the CAG promoter (human cytomegalovirus immediate early promoter enhancer with chicken beta-actin/rabbit beta-globin hybrid promoter), displaying widespread expression of firefly luciferase (Cao *et al.*, 2004). These mice when intravenously injected with CHBT in the dorsal and/or lateral tail vein form D-luciferin and bioluminesce in tissues rich in D-cysteine. Optical imaging of this bioluminescence using a Xenogen IVIS 200 imager enables the *in vivo* optical imaging of endogenous D-cysteine. *In vivo* optical imaging indicates relatively high levels of D-cysteine in the eyes and a transverse thoraco-abdominal organ [Figure 7]. Preliminary dissections localize the signal from the transverse thoraco-abdominal organ to the pancreas, stomach, and duodenum [Figure 8A]. Improved dissection of the pancreas from the stomach and duodenum, indicates that D-cysteine is present exclusively in the pancreas, and that the signal from the stomach and duodenum arise from residual pancreatic tissue surrounding the stomach and duodenum left behind during dissection [Figure 8B].

Impaired vision in serine racemase knockout mice

Candidate enzymes for the endogenous biosynthesis and degradation of mammalian D-cysteine are serine racemase and D-amino acid oxidase respectively. In fact, it would not be surprising at all if serine racemase indeed functioned as a cysteine racemase, given the close structural similarity between serine and cysteine. Namely, substituting an oxygen

atom in serine for a sulfur atom in cysteine. Interestingly, serine racemase knockout mice ($SR^{-/-}$) develop an age-related vision impairment, despite there being no statistical difference between D-serine levels in the eyeballs of WT and $SR^{-/-}$ mice (Horio *et al.*, 2011). The D-serine concentration in the eyeballs is under ~8 μM, relatively low compared to hippocampal D-serine levels of ~260 μM (Horio *et al.*, 2011). On the other hand, the eyes have relatively high levels of D-cysteine compared to other organs [Figures 7, 8]. All this indicates that the likely culprit for the age-related vision impairment in $SR^{-/-}$ mice is D-cysteine deficiency.

Electroretinogram responses are attenuated in older $SR^{-/-}$ compared to age-matched WT controls [Figure 9]. Both scotopic a-waves [Figure 9A] and b-waves [Figure 9B] are severely attenuated in older $SR^{-/-}$ compared to age-matched WT controls. Photopic b-waves are also diminished in older $SR^{-/-}$ compared to age-matched WT controls [Figure 9C]. Electroretinogram responses are unaffected in D-amino acid oxidase knockout mice ($DAAOx^{-/-}$) compared to age-matched WT controls [Figure 10]. Both scotopic b-waves [Figure 10A] and photopic b-waves [Figure 10B] are unaffected in $DAAOx^{-/-}$ compared to age-matched WT controls. The responses of the $DAAOx^{-/-}$ mice are slightly better.

Retinal fundus photography indicates focal disruptions of the retinal pigmented epithelium in older $SR^{-/-}$ mice [Figure 11]. In toluidine blue-stained retinal sections, overall thinning of the retina is observed in older $SR^{-/-}$ compared to WT [Figure 12]. The retinal ganglion cell layer (RGL) is a densely packed mono- and sometimes bi-layer in WT versus an interrupted monolayer in $SR^{-/-}$. The inner plexiform layer (IPL) is densely staining blue in WT versus grey in $SR^{-/-}$. The inner nuclear layer (INL), in WT the

cytoplasm is homogenously stained with nuclei, in $SR^{-/-}$ the cytoplasm is heterogeneously stained, which might indicate vacuolation, with very densely staining swollen and pyknotic nuclei. The outer plexiform layer is densely staining blue in WT versus grey in $SR^{-/-}$. The outer nuclear layer (ONL) in $SR^{-/-}$, compared to WT, is less organized. The inner segment (IS) and outer segment (OS) in $SR^{-/-}$, compared to WT, is less organized. The Bruch's membrane seems thickened in $SR^{-/-}$, compared to WT.

Chapter 1c. Discussion

Using HPLC we establish the presence of endogenous mammalian D-cysteine [Figure 4]. The luciferase bioluminescence assay for the stereo-specific detection of D-cysteine forms the biochemical basis for the *in vivo* optical imaging of endogenous D-cysteine in transgenic mice overexpressing firefly luciferase. The assay readily detects 0.1-1.0 μM D-cysteine [Figure 6], and so is suitable for monitoring physiologic mammalian tissue levels of D-cysteine. *In vivo* optical imaging of endogenous D-cysteine in transgenic mice overexpressing firefly luciferase indicates relatively high levels of D-cysteine in the eyes and a transverse thoraco-abdominal organ [Figure 7], which on dissection is identified as the pancreas [Figure 8].

Serine racemase is a candidate enzyme for the endogenous biosynthesis of mammalian D-cysteine. In fact, it would not be surprising at all if serine racemase indeed functioned as a cysteine racemase, given the close structural similarity between serine and cysteine. Namely, substituting an oxygen atom in serine for a sulfur atom in cysteine. Consistent with the notion that serine racemase functions as a cysteine racemase, is that in a [cysteine]:[serine] 2:1 molar ratio, either L-cysteine or D-cysteine markedly inhibit (>90%) the *in vitro* racemization of L-serine by recombinant mouse serine racemase (Panizzutti *et al.*, 2001). Cysteine is the only amino acid whose D-enantiomer and L-enantiomer both inhibit serine racemization, consistent with cysteine also being a substrate of serine racemase and therefore acts as a competitive inhibitor for serine racemization. If both serine and cysteine are equally good serine racemase substrates, one would expect that at a [cysteine]:[serine] 2:1 molar ratio, cysteine to

inhibit serine racemization by 67%. Since the observed inhibition is >90% (Panizzutti *et al.*, 2001), this suggests that cysteine is a substantially better serine racemase substrate than serine itself.

Interestingly, $SR^{-/-}$ mice develop an age-related vision impairment [Figures 9-11], despite there being no statistical difference between D-serine levels in the eyeballs of WT and $SR^{-/-}$ mice (Horio *et al.*, 2011). Eye D-serine levels are ~8 μM, relatively merged compared to hippocampal levels of ~260 μM (Horio *et al.*, 2011). D-cysteine is enriched in the eyes [Figures 7, 8], making its deficiency likely responsible for the age-related vision impairment observed in $SR^{-/-}$ mice.

D-amino acid oxidase is a candidate enzyme for the endogenous degradation of mammalian D-cysteine. D-amino acid oxidase using FAD as a cofactor oxidizes D-amino acids into the corresponding imino acids, producing hydrogen peroxide and ammonia. It should be noted that it is erroneously reported that D-cysteine is a very poor substrate for D-amino acid oxidase (Man & Bada, 1987). The classic study which measured the oxidation rate of various D-amino acids by mammalian D-amino acid oxidase only attempted measurements on cystine, not cysteine (Bender & Krebs, 1950). The reason being, the D-amino acid oxidase activity assays are conducted in 0.1 M pyrophosphate buffer, pH 8.4; at such alkaline pH, the thiol group of cysteine is readily oxidized, particularly given that the air space of the assay flasks are flushed with oxygen. Since D-amino acid oxidase is a peroxisomal enzyme, it would be most relevant to assay its activity at the physiologic pH of peroxisomes, pH 6.9-7.1 (Jankowski *et al.*, 2001), where the thiol group of cysteine is not readily oxidized. Most D-amino acid oxidase activity assays rely on detecting the hydrogen peroxide produced, however this is unsuitable

when cysteine is the substrate, because the free thiol on cysteine would react with hydrogen peroxide quenching the signal. A relatively recent study (Bailey *et al.*, 2015) assessed *in vitro* the ability of porcine D-amino acid oxidase to degrade D-cysteine in pH 7.4 tris reaction buffer by bioluminescently assaying the decrease in D-cysteine levels. Their results not only indicate that D-cysteine is a substrate for D-amino acid oxidase, but it also suggests that D-cysteine is a substantially better substrate for D-amino acid oxidase than D-serine, since an equimolar amount of D-serine did not inhibit D-cysteine degradation by D-amino acid oxidase.

LEA/SENDAI rats, a mutant rat strain naturally lacking D-amino acid oxidase (Konno *et al.*, 2009), develop nonobese type 2 diabetes mellitus (Okamura *et al.*, 2013). This is particularly interesting given that the pancreas is a D-cysteine rich organ [Figure 8].

Chapter 1d. Methods

High-performance liquid chromatography

Mouse liver is homogenized in 10X Hepes-NaOH buffer, pH 7.7, using a tissue grinder at 4 ºC for 5 minutes, spun at maximum speed in 4 ºC microfuge for 30 minutes, supernatant is used in subsequent steps. Proteins are precipitated by bringing supernatant to 2 N HCl using 10 N HCl. Alternatively, mouse liver is extracted directly in 1X 2 N HCl for 10 minutes at 99 ºC. In either case, samples are cooled on ice for 30 minutes, then spun at maximum speed in 4 ºC microfuge for 30 minutes, supernatant is then used in subsequent steps. To fluorescently label thiols, supernatant is neutralized by adding one equivalent NaOH using 10 N NaOH, followed right away with 1 volume equivalent of 1.0 mM ABD-F in 0.2 mM sodium borate buffer, pH 8.0, 1 mM EDTA and 0.1 volume equivalent of 10% tri-*n*-butyl phosphine in acetonitrile. The mixture is incubated at 50 ºC for 5 minutes in thermomixer with simultaneous mixing at maximum speed, then placed on ice, and mixed with 0.01 volume equivalent of 2.5 N HCl. The fluorescently labeled samples are separated using HPLC on a chiral crown ether column, CROWNPAK® CR (+) (150 × 4 mm i.d., 5 µM; Chiral Technologies Inc., Wester Chester, Pennsylvania). A mobile phase of pH 1.0 aqueous perchloric acid (1 ml/min flow rate) is used for isocratic elution. The fluorescent ABD adducts are detected using fluorescence at an emission wavelength of 510 nm following excitation at 380 nm.

The luciferase bioluminescence assay for the stereo-specific detection of D-cysteine.

D-cysteine standards are made in 1X Reaction buffer [500-mM Tricine buffer (89.6 g/l), pH 7.8, 100-mM $MgSO_4$ (12 g/l), and 2-mM EDTA (0.832 g/l)], 1 % Triton X-100, 1-mM TCEP, 0.1 mg/ml 2-cyano-6-hydroxybenzothiazole, and 200-mM potassium carbonate. Wherever possible nitrogen bubbled sterile water is used to prepare the solutions. Samples are incubated at 30 ºC for 10 minutes in thermomixer with simultaneous mixing at maximum speed. Then left to cool on ice for 5 minutes. 20-µl of 10-N HCl is then added to neutralize the alkalinity of the potassium carbonate. The following are diluted in the final solution before measurements: 10X 50-mM Mg-ATP in 10-mM Tris pH 7.8, and 500X 2-µl of firefly luciferase 1 mg/ml stock solution in 25-mM Tris-acetate (3 g/l Tris base; 0.6 g/l glacial acetic acid), pH 7.8, 0.2-M ammonium sulfate (26.4 g/l), 15% (v/v) glycerol and 30% (v/v) ethylene glycol; these can be premixed and added together. Bioluminescence is then measured at 560 nm.

***In vivo* optical imaging of endogenous D-cysteine in transgenic mice overexpressing firefly luciferase.**

FVB-Tg(CAG-luc,-GFP)L2G85Chco/J male mice are anesthetized using isofluorane inhalation anesthesia. The mice are then sterile injected intravenously in the dorsal and/or lateral tail vein with CHBT (0.5 µmol in 50 µl of 1:1 DMSO:PBS). *In vivo* bioluminescence is imaged using a Xenogen IVIS 200 optical imager before and after dissection.

Electroretinograms (ERGs)

An Espion electroretinogram (ERG) Diagnosys machine (Diagnosys LLC, Littleton, MA, USA) is used to record the ERGs. For scotopic recordings, mice are dark adapted overnight, and for photopic recordings, mice are adapted to background white light at an intensity of 30 cd/m^2 for 10 min. The mice are anesthetized with an intraperitoneal injection of ketamine hydrochloride (100 mg/kg body weight; Fort Dodge Laboratories, Fort Dodge, IA) and xylazine (5 mg/kg body weight; Fort Dodge Laboratories, Fort Dodge, IA). The corneas are then anesthetized with topical administration of 0.5% proparacaine hydrochloride (Alcon Labs, Forth Worth, TX), pupils are then dilated with 1% tropicamide (Alcon Labs) and 2.5% phenylephrine (Bausch and Lomb, Tampa, FL). Platinum electrodes are then placed on each cornea after application of gonioscopic prism solution (Alcon Labs), and a reference electrode is placed subcutaneously in the anterior scalp between the eyes, and a ground electrode is inserted into the tail. Body temperature is maintained at 39 °C using a heating pad, light stimuli are provided by a Ganzfeld bowl illuminator ensuring equal illumination of the eyes, and responses from each eye are simultaneously recorded. Scotopic ERGs are recorded at 11 intensity levels of white light that ranged from −3.00 to 1.40 log cd-s/m^2. Photopic ERGs are recorded at three intensity levels of white light ranging from 0.60 to 1.40 log cd-s/m^2 with a 30 cd/m^2 background.

Retinal fundus photography

Mice are anesthetized by intraperitoneal injection of 120 µg/g body weight of ketamine hydrochloride (Fort Dodge Laboratories, Fort Dodge, IA). 0.5% tropicamide and 0.5%

phenylephrine are used to fully dilate the pupils, the fundus is then photographed with a fundus camera.

Light Microscopy

As previously described (Sarfare *et al.*, 2014) with the exception that transcardial perfusion is used to fix the whole mouse prior to enucleation of the eyes.

Chapter 1e. Tables

Table 1. ***In vitro*** **racemization half-lives of different amino acids (adapted with modifications from Man & Bada, 1987).**

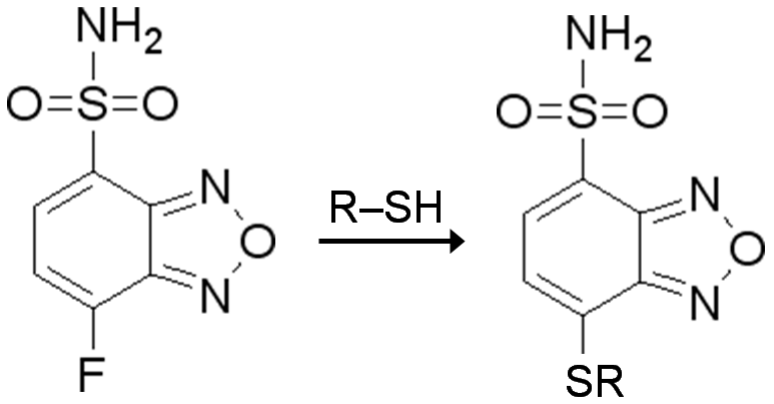
Amino acid	Half-life (days)
Cysteine	2
Serine	3
Threonine	20
Aspartic acid, Methionine	30
Lysine, Tryptophan	40
Phenyl alanine, Tyrosine	50
Alanine	120
Isoleucine, Leucine, Valine	300

Table 2. The retention times of various biological thiols derivatized with ABD-F separated using a CROWNPAK® CR (+) column containing a chiral crown ether.

Thiol	Retention time (min)
D-Cysteine	18.419
L-Cysteine	41.743
Cysteamine	49.768
D-Homocysteine	68.725
L-Homocysteine	209.550
L-Glutathione	260.672

Chapter 1f. Figures

Figure 1.

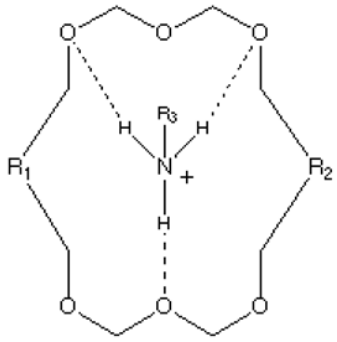


ABD-F **ABD-SR**

Figure 1. Structure of ABD-F and its thiol adduct.

Figure 2.

A



B

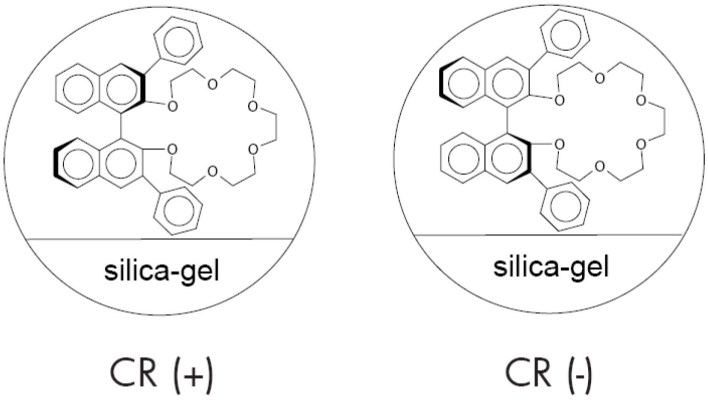


Figure 2. Structure of the chiral crown ether present in the CROWNPAK® column.

(**A**) Under acidic conditions the amino group is protonated to form an ammonium ion which complex with the chiral crown ether inside the chiral cavity of the crown ether. This enables the chiral crown ether to act as a chiral selector for compounds with a primary amino group near their chiral center. (**B**) The absolute configuration is inferred from the elution order. In CR (+) the D-stereoisomer elutes before the L-stereoisomer. CR (-) has reverse enantioselectivity and therefore provides the reverse order of elution relative to the CR (+), i.e., the L-stereoisomer elutes before the D-stereoisomer.

Figure 3.

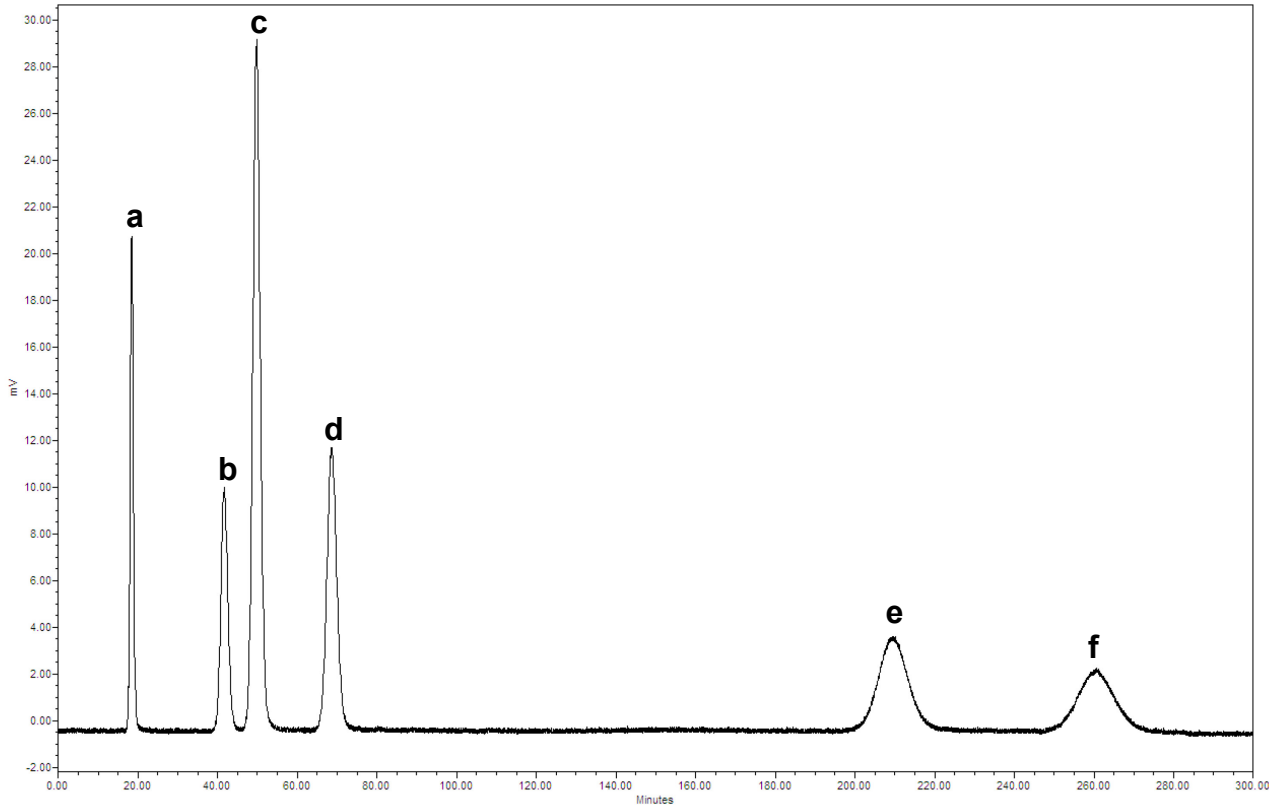

a
b
c
d
e
f
mV
Minutes
30.00
28.00
26.00
24.00
22.00
20.00
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
-2.00
0.00
20.00
40.00
60.00
80.00
100.00
120.00
140.00
160.00
180.00
200.00
220.00
240.00
260.00
280.00
300.00

Figure 3. Chromatogram of commonly occurring biological thiols derivatized with ABD-F.

(**a**) 1 nmol ABD-D-cysteine, (**b**) 1 nmol ABD-L-cysteine, (**c**) 1 nmol ABD-cysteamine, (**d**) 1 nmol ABD-L-homocysteine, (**e**) 1 nmol ABD-D-homocysteine, (**f**) 1 nmol ABD-glutathione; column, CROWNPAK® CR (+) column 150 × 4 mm i.d., 5 μM; isocratic eluent, pH 1.0 aqueous perchloric acid; flow rate, 1 ml/min; detection, Ex 380 nm, Em 510 nm.

Figure 4.

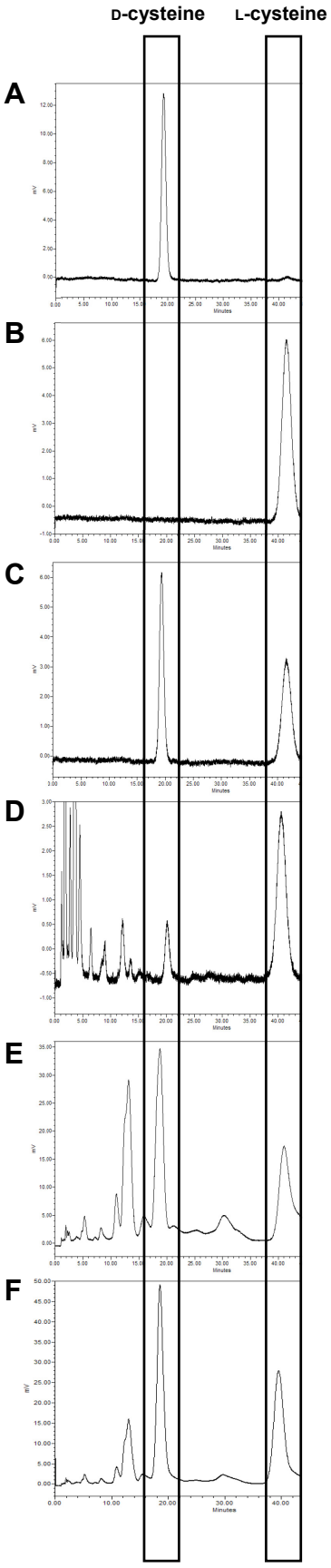


Figure 4. HPLC separation of fluorescent ABD-F adducts of D-cysteine and L-cysteine using a CROWNPAK® CR (+) column containing a chiral crown ether.

(**A**) 1 nmol D-cysteine standard. (**B**) 1 nmol L-cysteine standard. (**C**) 1 nmol racemic cysteine mixture standard. (**D**) Mouse liver lysate made by homogenizing the liver in Hepes-NaOH buffer, pH 7.7, using a tissue grinder. The D:L ratio of cysteine is 1:6. (**E**) Mouse live extract made by lysing the liver in 1X 2 N HCl for 10 minutes at 99 ºC. The D:L ratio of cysteine is 1:1. (**F**) The sample in **E** spiked with a racemic cysteine mixture, this augments both the D-cysteine and L-cysteine peaks confirming their identity.

Figure 5.

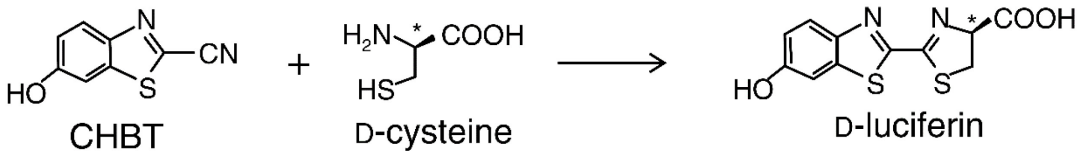


Figure 5. Chemical synthesis of D-luciferin.

The chiral carbon, marked with an asterisk, of luciferin is from cysteine (adapted with modification from Niwa *et al.*, 2006). This forms the basis of the luciferase bioluminescence assay for the stereo-specific detection of D-cysteine.

Figure 6.

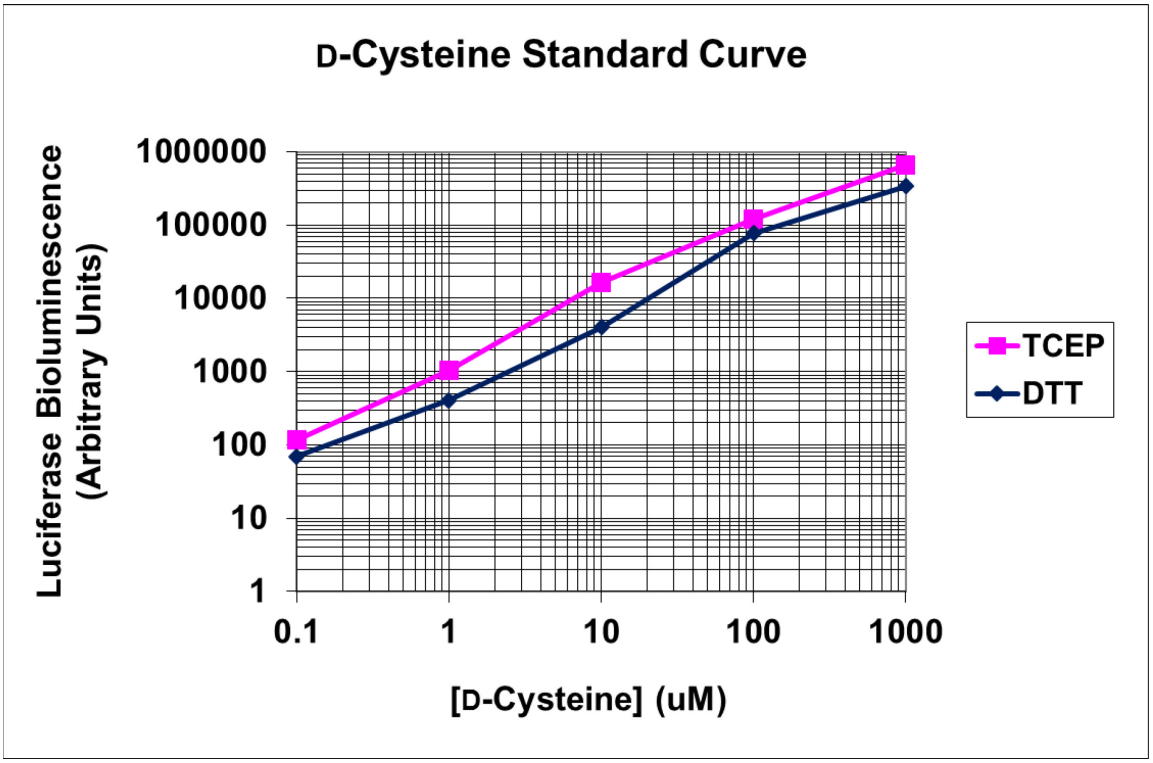

D-Cysteine Standard Curve
Luciferase Bioluminescence (Arbitrary Units)
1000000
100000
10000
1000
100
10
1
0.1
1
10
100
1000
[D-Cysteine] (uM)
TCEP
DTT

Figure 6. Sensitivity of the luciferase bioluminescence assay for the stereo-specific detection of D-cysteine.

The assay readily detects 0.1-1.0 μM D-cysteine. Using TCEP as a reducing agent provides better signal than DTT.

Figure 7.

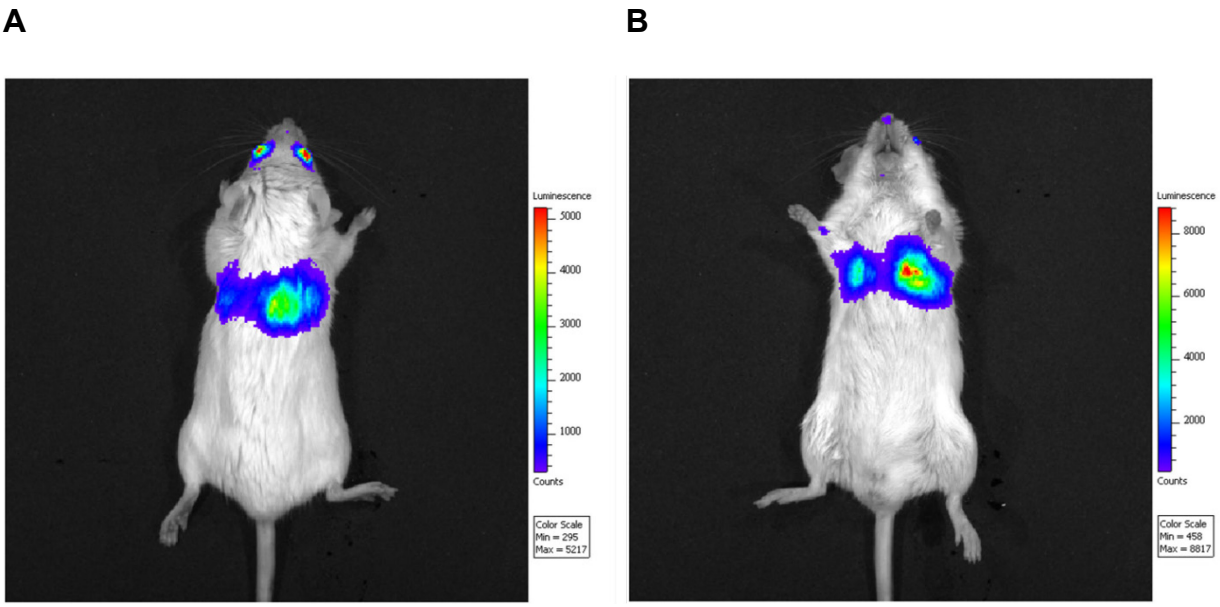

A
B
Luminescence
5000
4000
3000
2000
1000
Counts
Color Scale
Min = 295
Max = 5217
Luminescence
8000
6000
4000
2000
Counts
Color Scale
Min = 458
Max = 8817

Figure 7. *In vivo* optical imaging of endogenous D-cysteine in transgenic mice overexpressing firefly luciferase.

(**A**) Prone position. (**B**) Supine position. *In vivo* optical imaging indicates relatively high levels of D-cysteine in the eyes and a transverse thoraco-abdominal organ. $Luc^{+/+}$ male mice are anesthetized by isoflurane inhalation anesthesia and sterile injected intravenously in the dorsal and/or lateral tail vein with CHBT (0.5 μmol in 50 μl of 1:1 DMSO:PBS). *In vivo* bioluminescence was imaged using a Xenogen IVIS 200 optical imager.

Figure 8.

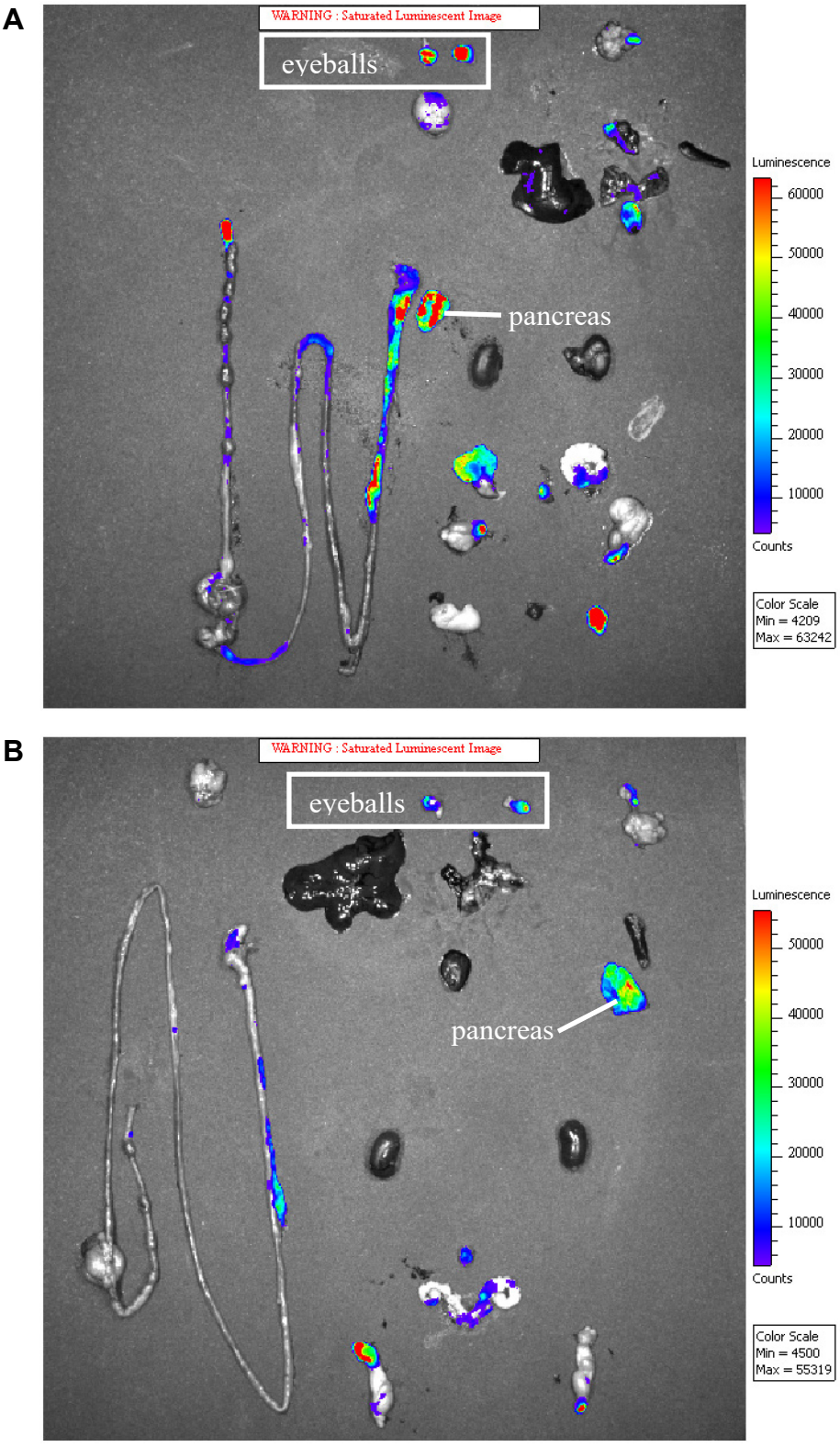

A
WARNING : Saturated Luminescent Image
eyeballs
pancreas
Luminescence
60000
50000
40000
30000
20000
10000
Counts
Color Scale
Min = 4209
Max = 63242
B
WARNING : Saturated Luminescent Image
eyeballs
pancreas
Luminescence
50000
40000
30000
20000
10000
Counts
Color Scale
Min = 4500
Max = 55319

Figure 8. Optical imaging of endogenous D-cysteine in dissected transgenic mice overexpressing firefly luciferase, identifies the transverse thoraco-abdominal organ rich in D-cysteine as the pancreas.

(**A**) Preliminary dissections localize the signal from the transverse thoraco-abdominal organ to the pancreas, stomach, and duodenum. (**B**) Improved dissection of the pancreas from the stomach and duodenum, indicates that D-cysteine is present exclusively in the pancreas, and that the signal from the stomach and duodenum arise from residual pancreatic tissue surrounding the stomach and duodenum left behind during dissection.

Figure 9.

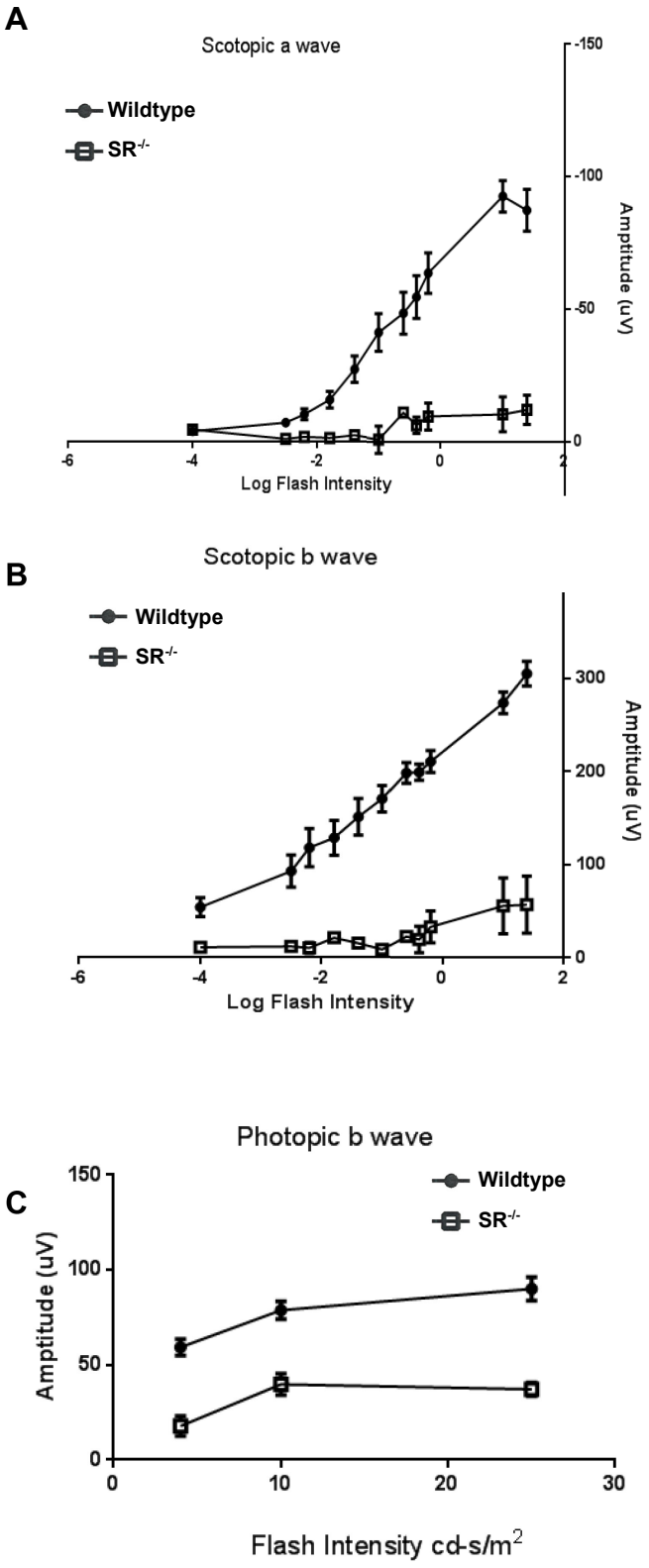

A
Scotopic a wave
Wildtype
SR-/-
-150
-100
-50
0
Amplitude (uV)
-6
-4
-2
0
2
Log Flash Intensity
B
Scotopic b wave
Wildtype
SR-/-
300
200
100
0
Amplitude (uV)
-6
-4
-2
0
2
Log Flash Intensity
C
Photopic b wave
Wildtype
SR-/-
150
100
50
0
Amplitude (uV)
0
10
20
30
Flash Intensity cd-s/m2

Figure 9. Electroretinogram responses are attenuated in $SR^{-/-}$ compared to age-matched WT controls.

(**A**) Scotopic a-waves and (**B**) b-waves are severely attenuated in $SR^{-/-}$ compared to age-matched WT controls. (**C**) Photopic b-waves are also diminished in $SR^{-/-}$ compared to age-matched WT controls. All mice were 15 months of age.

Figure 10.

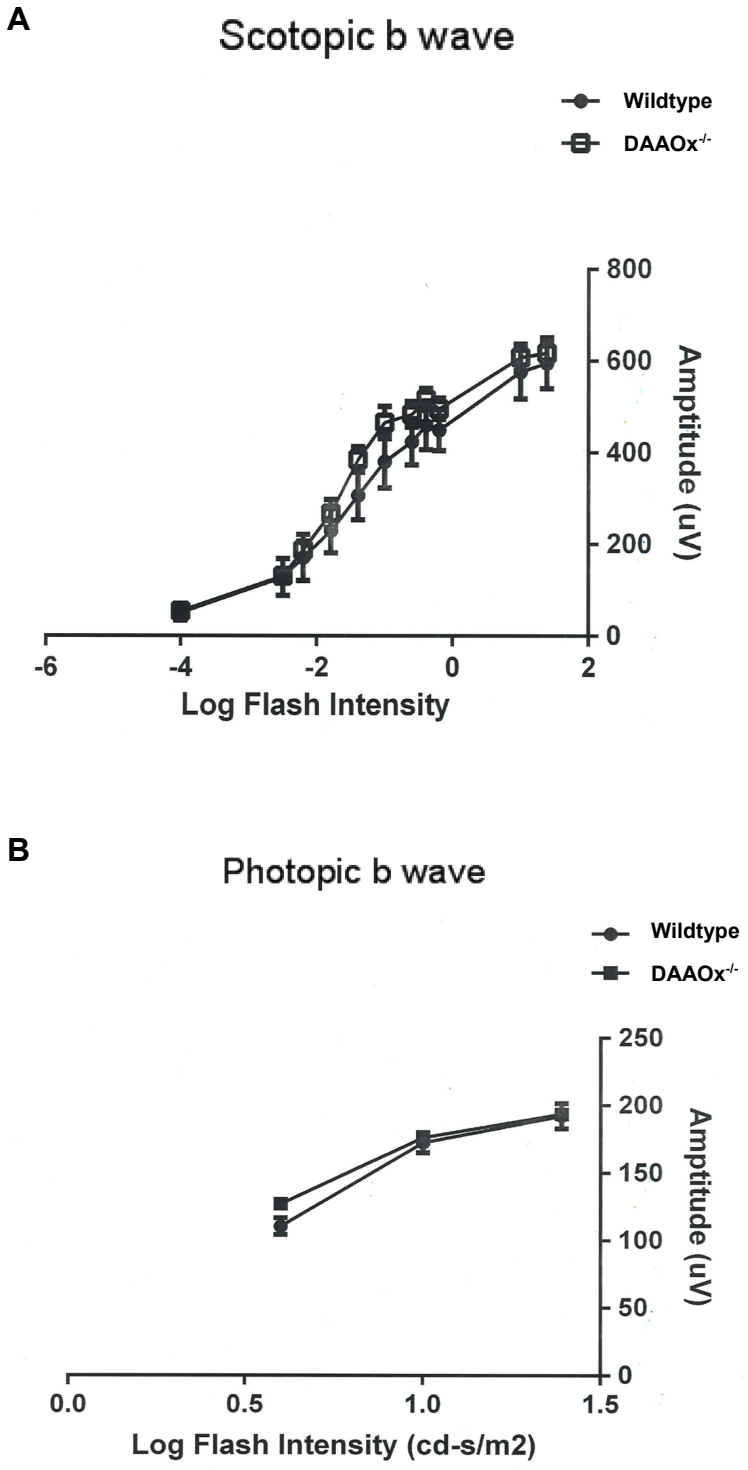

A
Scotopic b wave
Wildtype
DAAOx-/-
800
600
400
200
0
Amptitude (uV)
-6
-4
-2
0
2
Log Flash Intensity
B
Photopic b wave
Wildtype
DAAOx-/-
250
200
150
100
50
0
Amptitude (uV)
0.0
0.5
1.0
1.5
Log Flash Intensity (cd-s/m2)

Figure 10. Electroretinogram responses are unaffected in DAAOx$^{-/-}$ compared to age-matched WT controls.

(**A**) Scotopic b-waves and (**B**) photopic b-waves are unaffected in DAAOx$^{-/-}$ compared to age-matched WT controls. The responses of the DAAOx$^{-/-}$ mice are slightly better. All mice were 17-18 months of age.

Figure 11.

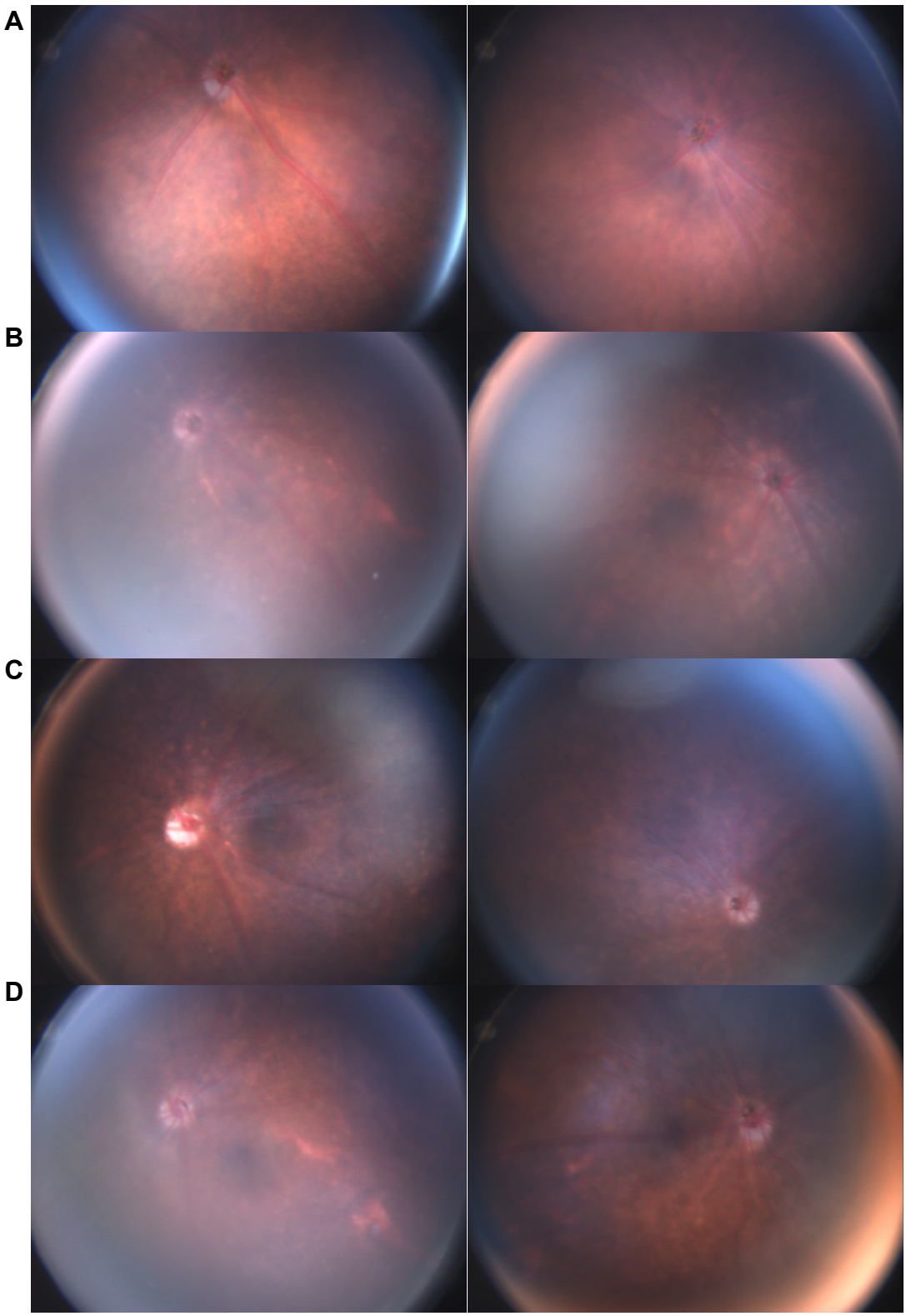


Figure 11. Retinal fundus photography indicates focal disruptions of the retinal pigmented epithelium in $SR^{-/-}$ mice.

(**A**) WT 14-month old mouse. (**C-D**) $SR^{-/-}$ 14-month old mice.

Figure 12.

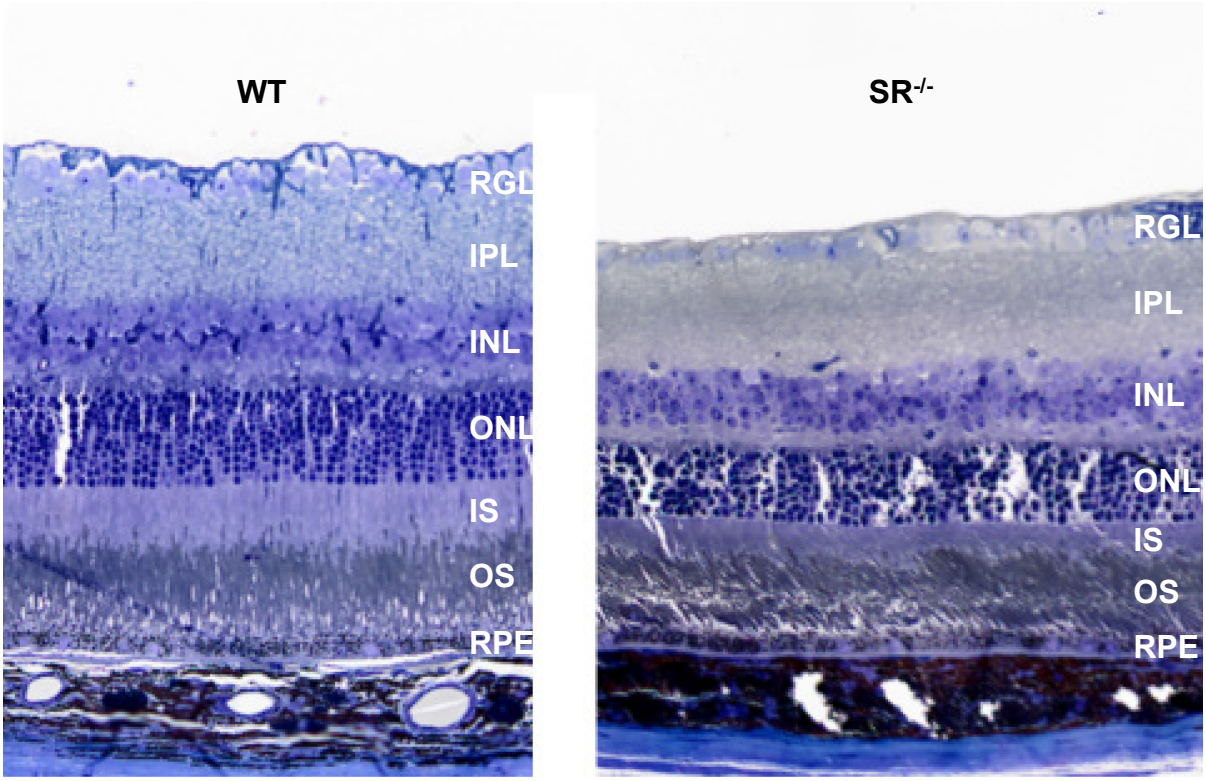


Figure 12. Toluidine blue-stained retinal sections of a 21-month old WT mouse and an age-matched $SR^{-/-}$ mouse.

Overall thinning of the retina is observed in $SR^{-/-}$ compared to WT. The retinal ganglion cell layer (RGL) is a densely packed mono- and sometimes bi-layer in WT versus an interrupted monolayer in $SR^{-/-}$. The inner plexiform layer (IPL) is densely staining blue in WT versus grey in $SR^{-/-}$. The inner nuclear layer (INL), in WT the cytoplasm is homogenously stained with nuclei, in $SR^{-/-}$ the cytoplasm is heterogeneously stained, which might indicate vacuolation, with very densely staining swollen and pyknotic nuclei. The outer plexiform layer is densely staining blue in WT versus grey in $SR^{-/-}$. The outer nuclear layer (ONL) in $SR^{-/-}$, compared to WT, is less organized. The inner segment (IS) and outer segment (OS) in $SR^{-/-}$, compared to WT, is less organized. The Bruch's membrane seems thickened in $SR^{-/-}$, compared to WT.

Chapter 2. Conclusion

In conclusion, we establish the presence of endogenous mammalian D-cysteine by HPLC utilizing a chiral crown ether column as a chiral selector. Based on the fact that D-cysteine is used in the chemical synthesis of D-luciferin, the substrate for firefly luciferase, from 2-cyano-6-hydroxybenzothiazole, we develop a method for the *in vivo* optical imaging of endogenous D-cysteine in transgenic mice overexpressing firefly luciferase. Using this method, we find relatively high levels of D-cysteine in the eyes and a transverse thoraco-abdominal organ, which on dissection is identified as the pancreas. We identify serine racemase and D-amino acid oxidase as candidate enzymes for the endogenous biosynthesis and degradation of mammalian D-cysteine respectively. We find that serine racemase knockout mice develop an age-related vision impairment.

Chapter 3. References

Bada, J. L. (1984) *Methods Enzymol.* **106**, 98-115. *In vivo* racemization in mammalian proteins.

Bailey, T. S., Donor, M. T., Naughton, S. P., & Pluth, M.D. (2015) *Chem. Commun. (Camb.)* **51**, 5425-5428. A simple bioluminescent method for measuring D-amino acid oxidase activity.

Basu, A. C., Tsai, G. E., Ma, C. -L., Ehmsen, J. T., Mustafa, A. K., Han, L., Jiang, Z. I., Benneyworth, M. A., Froimowitz, M. P., Lange, N., Snyder, S. H., Bergeron, R., & Coyle, J. T. (2008) *Mol. Psychiatry* **14**, 719-727. Targeted disruption of serine racemase affects glutamatergic neurotransmission and behavior.

Bender, A. E., & Krebs, H. A. (1950) *Biochem. J.* **46**, 210-219. The oxidation of various α-amino-acids by mammalian D-amino-acid oxidase, L-amino-acid oxidase of cobra venom and the L- and D-amino-acid oxidases of *Neurospora crassa*.

Blackmond, D. G. (2010) *Cold Spring Harb. Perspect. Biol.* **2**, a002147. The origin of biological homochirality.

Corrigan, J. J. (1969) *Science* **164**, 142-149. D-amino acids in animals.

D'Aniello, A., & Giuditta, A. (1978) *J. Neurochem.* **31**, 1107-1108. Presence of D-aspartate in squid axoplasm and in other regions of the cephalopod nervous system.

Dunlop, D. S., Neidle, A., McHale, D., Dunlop, D. M., & Lajtha, A. (1986) *Biochem. Biophys. Res. Commun.* **141**, 27-32. The presence of free D-aspartic acid in rodents and man.

Dunlop, D. S., & Neidle, A. (1997) *Biochem. Biophys. Res. Commun.* **235**, 26-30. The origin and turnover of D-serine in brain.

Efeyan, A., Comb, W. C., & Sabatini, D. M. (2015) *Nature* **517**, 302-310. Nutrient-sensing mechanisms and pathways.

Ehmsen, J. T., Ma, T. M., Sason, H., Rosenberg, D., Ogo, T., Furuya, S., Snyder, S. H., & Wolosker, H. (2013) *J. Neurosci.* **33**, 12464-12469. D-serine in glia and neurons derives from 3-phosphoglycerate dehydrogenase.

Felbeck, H. (1985) *J. Exp. Zool.* **234**, 145-149. Occurrence and metabolism of D-aspartate in the gutless bivalve *Solemya reidi*.

Felbeck, H., & Wiley, S. (1987) *Biol. Bull.* **173**, 373-378. Free D-amino acids in the tissues of marine bivalves.

Fujii, K., Maeda, K., Hikida, T., Mustafa, A. K., Balkissoon, R., Xia, J., Yamada, T., Ozeki, Y., Kawahara, R., Okawa, M., Huganir, R. L., Ujike, H., Snyder, S. H., & Sawa, A. (2006) *Mol. Psychiatry* **11**, 150-157. Serine racemase binds to PICK1: Potential relevance to schizophrenia.

Gal, J. (2011) *Chirality* **23**, 1-16. Louis Pasteur, language, and molecular chirality. I. Background and dissymmetry.

Hashimoto, A., Nishikawa, T., Hayashi, T., Fujii, N., Harada, K., Oka, T., & Takahashi, K. (1992a) *FEBS Lett.* **296**, 33-36. The presence of free D-serine in rat brain.

Hashimoto, A., Nishikawa, T., Oka, T., Takahashi, K., & Hayashi, T. (1992b) *J. Chromatogr.* **582**, 41-48. Determination of free amino acid enantiomers in rat brain and serum by high-performance liquid chromatography after derivatization with N-*tert*.-butyloxycarbonyl-L-cysteine and *o*-phthaldialdehyde.

Hashimoto, A., Nishikawa, T., Oka, T., & Takahashi, K. (1993) *J. Neurochem.* **60**, 783-786. Endogenous D-serine in rat brain: *N*-methyl-D-aspartate receptor-related distribution and aging.

Helboe, L., Egebjerg, J., Møller, M., & Thomsen, C. (2003) *Eur. J. Neurosci.* **18**, 2227-2238. Distribution and pharmacology of alanine-serine-cysteine transporter 1 (asc-1) in rodent brain.

Helfman, P. M., & Bada, J. L. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 2891-2894. Aspartic acid racemization in tooth enamel from living humans.

Helfman, P. M., & Bada, J. L. (1976) *Nature* **262**, 279-281. Aspartic acid racemisation in dentine as a measure of ageing.

Hikida, T., Mustafa, A. K., Maeda, K., Fujii, K., Barrow, R. K., Saleh, M., Huganir, R. L., Snyder, S. H., Hashimoto, K., & Sawa, A. (2008) *Biol. Psychiatry* **63**, 997-1000. Modulation of D-serine levels in brains of mice lacking PICK1.

Horio, M., Kohno, M., Fujita, Y., Ishima, T., Inoue, R., Mori, H., & Hashimoto, K. (2011) *Neurochem. Int.* **59**, 853-859. Levels of D-serine in the brain and peripheral organs of serine racemase (*Srr*) knock-out mice.

Huang, A. S., Beigneux, A., Weil, Z. M., Kim, P. M., Molliver, M. E., Blackshaw, S., Nelson, R. J., Young, S. G., & Snyder, S. H. (2006) *J. Neurosci.* **26**, 2814-2819. D-aspartate regulates melanocortin formation and function: Behavioral alterations in D-aspartate oxidase-deficient mice.

Jankowski, A., Kim, J. H., Collins, R. F., Daneman, R., Walton, P., Grinstein, S. (2001) *J. Biol. Chem.* **276**, 48748-48753. *In situ* measurements of the pH of mammalian peroxisomes using the fluorescent protein pHluorin.

Kajiyama, N. (2002) Patent No.: US 6,376,208 B1. Method and reagent for quantitating D-cysteine.

Kauffman, G. B., & Myers, R. D. (1998) *Chem. Educator* **3**, 1. Pasteur's resolution of racemic acid: A sesquicentennial retrospect and a new translation.

Kim, P. M., Aizawa, H., Kim, P. S., Huang, A. S., Wickramasinghe, S. R., Kashani, A. H., Barrow, R. K., Huganir, R. L., Ghosh, A., & Snyder, S. H. (2005) *Proc. Natl. Acad. Sci. USA* **102**, 2105-2110. Serine racemase: Activation by glutamate neurotransmission via glutamate receptor interacting protein and mediation of neuronal migration.

Konno, R., Okamura, T., Kasai, N., Summer, K. H., & Niwa, A. (2009) *Amino Acids* **37**, 367-375. Mutant rat strain lacking D-amino-acid oxidase.

Krebs, H. A. (1935) *Biochem. J.* **29**, 1620-1644. Metabolism of amino acids: Deamination of amino acids.

Ma, T. M., Abazyan, S., Abazyan, B., Nomura, J., Yang, C., Seshadri, S., Sawa, A., Snyder, S. H., & Pletnikov, M. V. (2013) *Mol. Psychiatry* **18**, 557-567. Pathogenic disruption of DISC1-serine racemase binding elicits schizophrenia-like behavior via D-serine depletion.

Ma, T. M., Paul, B. D., Fu, C., Hu, S., Zhu, H., Blackshaw, S., Wolosker, H., & Snyder, S. H. (2014) *J. Biol. Chem.* **289**, 29631-29641. Serine racemase regulated by binding to stargazin and PSD-95: Potential *N*-methyl-D-aspartate-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (NMDA-AMPA) glutamate neurotransmission cross-talk.

Man, E. H., & Bada, J. L. (1987) *Ann. Rev. Nutr.* **7**, 209-225. Dietary D-amino acids.

Matsui, T., Sekiguchi, M., Hashimoto, A., Tomita, U., Nichikawa, T., & Wada, K. (1995) *J. Neurochem.* **65**, 454-458. Functional comparison of D-serine and glycine in rodents: The effect on cloned NMDA receptors and the extracellular concentration.

Matsuo, H., Kanai, Y., Tokunaga, M., Nakata, T., Chairoungdua, A., Ishimine, H., Tsukada, S., Ooigawa, H., Nawashiro, H., Kobayashi, Y., Fukuda, J., & Endou, H. (2004) *Neurosci. Lett.* **358**, 123-126. High affinity D- and L-serine transporter Asc-1: Cloning and dendritic localization in the rat cerebral and cerebellar cortices.

Mothet, J. P., Parent, A. T., Wolosker, H., Brady, R. O., Jr., Linden, D. J., Ferris, C. D., Rogawski, M. A., & Snyder, S. H. (2000) *Proc. Natl. Acad. Sci. USA* **97**, 4926-4931. D-serine is an endogenous ligand for the glycine site of the *N*-methyl-D-aspartate receptor.

Mustafa, A. K., Kim, P. M., & Snyder, S. H. (2004) *Neuron Glia Biol.* **1**, 275-281. D-Serine as a putative glial neurotransmitter.

Mustafa, A. K., Kumar, M., Selvakumar, B., Ho, G. P., Ehmsen, J. T., Barrow, R. K., Amzel, L. M., & Snyder, S. H. (2007) *Proc. Natl. Acad. Sci. USA* **104**, 2950-2955. Nitric oxide *S*-nitrosylates serine racemase, mediating feedback inhibition of D-serine formation.

Mustafa, A. K., van Rossum, D. B., Patterson, R. L., Maag, D., Ehmsen, J. T., Gazi, S. K., Chakraborty, A., Barrow, R. K., Amzel, L. M., & Snyder, S. H. (2009) *Proc. Natl. Acad. Sci. USA* **106**, 2921-2926. Glutamatergic regulation of serine racemase via reversal of PIP2 inhibition.

Mustafa, A. K., Ahmad, A. S., Zeynalov, E., Gazi, S. K., Sikka, G., Ehmsen, J. T., Barrow, R. K., Coyle, J. T., Snyder, S. H., & Doré, S. (2010) *J. Neurosci.* **30**, 1413-1416. Serine racemase deletion protects against cerebral ischemia and excitotoxicity.

Niwa, K., Nakamura, M., & Ohmiya, Y. (2006) *FEBS Lett.* **580**, 5283-5287. Stereoisomeric bio-inversion key to biosynthesis of firefly D-luciferin.

Okamura, T., Pei, X. Y., Miyoshi, I., Shimizu, Y., Takanashi-Yanobu, R., Mototani, Y., Kanai, T., Satoh, J., Kimura, N., & Kasai, N. (2013) *J. Diabetes Res.* **2013**, 986462. Phenotypic characterization of LEA rat: A new rat model of nonobese type 2 diabetes.

Panizzutti, R., De Miranda, J., Ribeiro, C. S., Engelender, S., & Wolosker, H. (2001) *Proc. Natl. Acad. Sci. USA* **98**, 5294-5299. A new strategy to decrease *N*-methyl-D-aspartate (NMDA) receptor coactivation: Inhibition of D-serine synthesis by converting serine racemase into an eliminase.

Pasteur, L. (1848a) *Comptes rendus de l'Académie des sciences* (Paris) **26**, 535-538. Mémoire sur la relation qui peut exister entre la forme cristalline et la composition chimique, et sur la cause de la polarisation rotatoire.

Pasteur, L. (1848b) *Annales de Chimie et de Physique*, **24**, 442-459. Sur les relations qui peuvent exister entre la forme cristalline, la composition chimique et le sens de la polarisation rotatoire.

Pasteur, L. (1852) *Ann. Chem.* **82**, 324-335. Untersuchungen über asparaginsaürer und aepfelsäure.

Reavley, D. A., & Burge, R. E. (1972) *Adv. Microb. Physiol.* **7**, 1-81. Walls and membranes in bacteria.

Robinson, T. (1976) *Life Sci.* **19**, 1097-1102. D-amino acids in higher plants.

Rutter, A. R., Fradley, R. L., Garrett, E. M., Chapman, K. L., Lawrence, J. M., Rosahl, T. W., & Patel, S. (2007) *Eur. J. Neurosci.* **25**, 1757-1766. Evidence from gene knockout studies implicates Asc-1 as the primary transporter mediating D-serine reuptake in the mouse CNS.

Sarfare, S., McKeown, A. S., Messinger, J., Rubin, G., Wei, H., Kraft, T. W., & Pittler, S. J. (2014) *Cell Commun. Signal.* **12**, 67. Overexpression of rod photoreceptor glutamic acid rich protein 2 (GARP2) increases gain and slows recovery in mouse retina.

Schell, M. J., Molliver, M. E., & Snyder, S. H. (1995) *Proc. Natl. Acad. Sci. USA* **92**, 3948-3952. D-serine, an endogenous synaptic modulator: Localization to astrocytes and glutamate-stimulated release.

Snyder, S. H., & Kim, P. M. (2000) *Neurochem. Res.* **25**, 553-560. D-amino acids as putative neurotransmitters: Focus on D-serine.

Srinivasan, N. G., Corrigan, J. J., & Meister, A. (1965) *J. Biol. Chem.* **240**, 796-800. Biosynthesis of D-serine in the silkworm, *Bombyx mori*.

Toyo'oka, T., & Imai, K. (1984) *Anal. Chem.* **56**, 2461-2464. New fluorogenic reagent having halogenobenzofurazan structure for thiols: 4-(Aminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole.

Uo, T., Yoshimura, T., Shimizu, S., & Esaki, N. (1998) *Biochem. Biophys. Res. Commun.* **246**, 31-34. Occurrence of pyridoxal 5′-phosphate-dependent serine raceamase in silkworm, *Bombyx mori*.

Weil, Z. M., Huang, A. S., Beigneux, A., Kim, P. M., Molliver, M. E., Blackshaw, S., Young, S. G., Nelson, R. J., & Snyder, S. H. (2006) *Behav. Brain Res.* **171**, 295-302. Behavioral alterations in male mice lacking the gene for D-aspartate oxidase.

Wolosker, H., Sheth, K. N., Takahashi, M., Mothet, J. P., Brady, R. O., Jr., Ferris, C. D., & Snyder S. H. (1999a) *Proc. Natl. Acad. Sci. USA* **96**, 721-725. Purification of serine racemase: Biosynthesis of the neuromodulator D-serine.

Wolosker, H., Blackshaw, S., & Snyder, S. H. (1999b) *Proc. Natl. Acad. Sci. USA* **96**, 13409-13414. Serine racemase: A glial enzyme synthesizing D-serine to regulate glutamate-*N*-methyl-D-aspartate neurotransmission.

Wolosker, H., & Radzishevsky, I. (2013) *Biochem. Soc. Trans.* **41**, 1546-1550. The serine shuttle between glia and neurons: Implications for neurotransmission and neurodegeneration.

Xie, X., Dumas, T., Tang, L., Brennan, T., Reeder, T., Thomas, W., Klein, R. D., Flores, J., O'Hara, B. F., Heller, H. C., & Franken, P. (2005) *Brain Res.* **1052**, 212-221. Lack of the alanine-serine-cysteine transporter 1 causes tremors, seizures, and early postnatal death in mice.

Yamanaka, M., Miyoshi, Y., Ohide, H., Hamase, K., & Konno, R. (2012) *Amino Acids* **43**, 1811-1821. D-amino acids in the brain and mutant rodents lacking D-amino acid oxidase.

Yamane, T., Miller, D. L., & Hopfield, J. J. (1981) *Biochemistry* **20**:7059-7063. Discrimination between D- and L-tyrosyl transfer ribonucleic acids in peptide chain elongation.

CURRICULUM VITAE

The Johns Hopkins University School of Medicine

Moataz [Moe] M. Gadalla — Name

May 28th, 2017 — Date of this version

Educational History:

M.D.-Ph.D. candidate		Program in Pharmacology and Molecular Sciences Mentor: Solomon H. Snyder, M.D.	Johns Hopkins School of Medicine
B.S.	2007	Major: Chemical and Biomolecular Engineering Minor: Biomedical Engineering	Cornell University
A.B.	2007	Double Major: Biological sciences, and Chemistry & Chemical Biology	Cornell University

Other Research Experience:

2006 Undergraduate Research Associate, Professor David A. Putnam Laboratory, Department of Chemical & Biomolecular Engineering, Cornell University, Ithaca, New York

2004–2007 Undergraduate Research Associate, Professor George P. Hess Laboratory, Field of Biochemistry, Molecular and Cell Biology, Department of Molecular Biology & Genetics, Cornell University, Ithaca, New York

Scholarships:

2005–2007 Hunter R. Rawlings III Cornell Presidential Research Scholar (RCPRS), Cornell University. $24,000 in Stipend and Research Support.

2005–2006 Cornell New Horizons Program Research Scholar, College of Veterinary Medicine, Cornell University. $18,000 in Stipend and Research Support.

2005 Cornell Howard Hughes Undergraduate Research Scholar, College of Arts & Sciences, Cornell University. $6,000 in Stipend and Research Support.

Honors and Awards:

2015 Selected to Attend the 65th Lindau Nobel Laureate Meeting, Interdisciplinary Meeting with Nobel Laureates from the fields of Physics, Physiology or Medicine, and Chemistry, Lindau and Mainau Island, Germany, June 28th-July 3rd, 2015, supported by the Oak Ridge Associated Universities (ORAU) Graduate Student Award and the Wyss Charitable Endowment.

2010 Scheinberg Travel Award, Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine

2007 *Summa Cum Laude*, Cornell University

2007 Hertz Fellowship Finalist, Hertz Foundation

2007 Baccalaureate Student Award, American Institute of Chemists

2007 Student Travel Award, Biophysical Society

2006 Undergraduate Student Travel Award, Office of Undergraduate Biology (OUB), College of Arts & Sciences, Cornell University

2006 Field of Pharmacology Graduate Poster Competition, Honorable Mention, College of Veterinary Medicine, Cornell University

2006 The 4th Annual Bioengineering Expo Poster Competition, 2nd Place, College of Engineering, Cornell University
2006 Phi Beta Kappa, College of Arts & Sciences, Cornell University
2005 Tau Beta Pi, the Engineering Honor Society, College of Engineering, Cornell University
2004 The National Society of Collegiate Scholars, Cornell University
2004 The Golden Key International Honor Society, Cornell University
2003–2007 Cornell Dean's Honor List, Cornell University

Peer Reviewed Articles:

Yuan, G., Vasavda, C., Peng, Y. J., Makerenko, V. V., Raghuraman, G., Nanduri, J., **Gadalla, M. M.**, Semenza, G. L., Kumar, G. K., Snyder, S. H., & Prabhakar, N. R. (2015) Protein kinase G-regulated production of H_2S governs oxygen sensing. *Sci. Signal.* 8:ra37.

Rao, F., Xu, J., Fu, C., Cha, J. Y., **Gadalla, M. M.**, Xu, R., Barrow, J. C., & Snyder, S. H. (2015) Inositol pyrophosphates promote tumor growth and metastasis by anatagonizing liver kinase B1. *Proc. Natl. Acad. Sci. USA* 112:1773-1778.

Rao, F., Xu, J., Khan, A. B., **Gadalla, M. M.**, Cha, J. Y., Xu, R., Tyagi, R., Dang, Y., Chakraborty, A., & Snyder, S. H. (2014) Inositol hexakisphosphate kinase-1 mediates assembly/disassembly of the CRL4–signalosome complex to regulate DNA repair and cell death. *Proc. Natl. Acad. Sci. USA* 111:16005-16010.

Peng, Y. J., Makarenko, V. V., Nanduri, J., Vasavda, C., Raghuraman, G., Yuan, G., **Gadalla, M. M.**, Kumar, G. K., Snyder, S. H., & Prabhakar, N. R. (2014) Inherent variations in CO-H_2S-mediated carotid body O_2 sensing mediate hypertension and pulmonary edema. *Proc. Natl. Acad. Sci. USA* 111:1174-1179.

Makarenko, V. V., Nanduri, J., Raghuraman, G., Fox, A. P., **Gadalla, M. M.**, Kumar, G. K., Snyder, S. H., & Prabhakar, N. R. (2012) Endogenous H_2S is required for hypoxic sensing by carotid body glomus cells. *Am. J. Physiol., Cell Physiol.* 303:C916-C923.

Morikawa, T., Kajimura, M., Nakamura, T., Hishiki, T., Nakanishi, T., Yukutake, Y., Nagahata, Y., Ishikawa, M., Hattori, K., Takenouchi, T., Takahashi, T., Ishii, I., Matsubara, K., Kabe, Y., Uchiyama, S., Nagata, E., **Gadalla, M.M.**, Snyder, S. H., Suematsu, M. (2012) Hypoxic regulation of the cerebral microcirculation is mediated by a carbon monoxide-sensitive hydrogen sulfide pathway. *Proc. Natl. Acad. Sci. USA* 109:1293-1298.

Sen, N., Paul, B. D., **Gadalla, M. M.**, Mustafa, A. K., Sen, T., Xu, R., Kim, S., & Snyder, S. H. (2012) Hydrogen sulfide-linked sulfhydration of NF-κB mediates its antiapoptotic actions. *Mol. Cell* 45:13-24.

Peng, Y. J., Nanduri, J., Raghuraman, G., Souvannakitti, D., **Gadalla, M. M.**, Kumar, G. K., Snyder, S. H., & Prabhakar, N. R. (2010) H_2S mediates O_2 sensing in the carotid body. *Proc. Natl. Acad. Sci. USA* 107:10719-10724.

Gadalla, M. M., & Snyder S. H. (2010) Hydrogen sulfide as a gasotransmitter. *J. Neurochem.* 113:14-26.

Mustafa, A. K., **Gadalla, M. M.**, Sen, N., Kim, S., Mu, W., Gazi, S. K., Barrow, R. K., Yang, G., Wang, R., & Snyder, S. H. (2009) H_2S signals through protein *S*-sulfhydration. *Sci. Signal.* 2:ra72.

Mustafa, A. K., **Gadalla, M. M.**, & Snyder S. H. (2009) Signaling by gasotransmitters. *Sci. Signal.* 2:re2.

Peer Reviewed Abstracts:

Sen, N., Paul, B., **Gadalla, M.**, Mustafa, A., Sen, T., Kim, S., & Snyder, S. Sulfhydration of p65 is anti-apoptotic. Program No. 106.14. 2011 Neuroscience Meeting Planner. Washington, D.C.: Society for Neuroscience, 2011. Online.

Vandiver, M. S., Sen, N., Paul, B., Xu, R., Ko, H. S., **Gadalla, M. M.**, Karuppagounder, S., Dawson, V., Dawson, T., & Snyder, S. H. *S*-sulfhydration of Parkin enhances its activity and protective function. Program No. 355.11. 2011 Neuroscience Meeting Planner. Washington, D.C.: Society for Neuroscience, 2011. Online.

Gadalla, M. M., & Snyder, S. H. Hydrogen sulfide as a gasotransmitter: A novel facile assay for *S*-sulfhydration. Program No. 614.23. 2010 Neuroscience Meeting Planner. San Diego, CA: Society for Neuroscience, 2010. Online.

Xu, R., **Gadalla, M. M.**, Vandiver, M. S., Paul, B. D., Chakraborty, A., Sen, N., Ma, T. M., Mustafa, A. K., & Snyder, S. H. Hydrogen sulfide causes *S*-sulfhydration of PTEN and decreases its activity. Program No. 133.21. 2010 Neuroscience Meeting Planner. San Diego, CA: Society for Neuroscience, 2010. Online.

Vandiver, M. S., Xu, R., Ko, H. S., **Gadalla, M. M.**, Dawson, V. L., Dawson, T. M., & Snyder, S. H. Hydrogen sulfide enhances parkin activity through protein *S*-sulfhydration. Program No. 655.20. 2010 Neuroscience Meeting Planner. San Diego, CA: Society for Neuroscience, 2010. Online.

Gadalla, M. M., & Hess, G. P. Discovery of a cocaine binding site on β-amyloid peptides which play an important rôle in Alzheimer's disease. 2007 Biophysical Society Meeting Abstracts. *Biophys. J.* Supplement, Abstract, 961-Pos.

Gadalla, M. M., & Hess, G. P. Cocaine binding site ligands of the nicotinic acetylcholine receptor regulate beta-amyloid peptide aggregation. Program No. 412. 2006 Neuroscience Meeting Planner. Atlanta, GA: Society for Neuroscience, 2006. Online.

Gadalla, M. M., & Hess, G. P. Discovery of a new regulatory site on beta-amyloid peptides. *Alzheimers Dement.* 2:S594.

Gadalla, M. M., Lewis, R. W., & Hess, G. P. Discovery of a regulatory cocaine-binding site on β-amyloid peptides. Pharmacology Graduate Poster Party, Cornell University, Ithaca, New York, June 17th, 2006.

Gadalla, M. M., & Hess, G. P. Unraveling the mystery—aptamers, the nicotinic acetylcholine receptor, and β-amyloid peptides. Cornell Engineering Alumni Association (CEAA) Conference Poster session, Cornell University, Ithaca, New York, April 21st, 2006.

Gadalla, M. M. Aptamers, the nicotinic acetylcholine receptor, and β-amyloid peptides—a mystery unraveled. Cornell Undergraduate Spring Research Forum, Cornell University, Ithaca, New York, April 19th, 2006.

Gadalla, M. M., & Hess, G. P. Discovery of a new regulatory site on β-amyloid peptides. The 4th Annual Bioengineering Expo, Institute of Biological Engineers, Cornell University, Ithaca, New York, March 1st, 2006.

Oral Presentations:

Gadalla, M. M., & Hess, G. P. *Cocaine binding site ligands of the nicotinic acetylcholine receptor regulate beta-amyloid peptide aggregation*. Neuroscience 2006, the 36th Annual Meeting of the Society for Neuroscience, Atlanta, Georgia, October 16th, 2006.

Gadalla, M. M. *Discovery of a cocaine-binding site on ß-amyloid peptides that regulates their aggregation*. Cornell Howard Hughes Scholars Program, Cornell University, Ithaca, New York, July 5th, 2006.

Gadalla, M. M. *Aptamers, the nicotinic acetylcholine receptor, and ß-amyloid peptides—a mystery unraveled*. Cornell Undergraduate Spring Research Forum, Cornell University, Ithaca, New York, April 19th, 2006.

Gadalla, M. M. *Understanding the mechanism of neuroreceptor transmission.* Cornell Howard Hughes Scholars Program, Cornell University, Ithaca, New York, July 20th, 2005.

Poster Presentations:

Sen, N., Paul, B., **Gadalla, M. M.**, Mustafa, A., Sen, T., Kim, S., & Snyder, S. H. Sulfhydration of p65 is anti-apoptotic. Neuroscience 2011, the 41st Annual Meeting of the Society for Neuroscience, Washington, D.C., November 12th, 2011.

Vandiver, M. S., Sen, N., Paul, B., Xu, R., Ko, H. S., **Gadalla, M. M.**, Karuppagounder, S., Dawson, V., Dawson, T., & Snyder, S. H. *S*-sulfhydration of Parkin enhances its activity and protective function. Neuroscience 2011, the 41st Annual Meeting of the Society for Neuroscience, Washington, D.C., November 14th, 2011.

Gadalla, M. M., & Snyder, S. H. Hydrogen sulfide as a gasotransmitter: A novel facile assay for *S*-sulfhydration. Neuroscience 2010, the 40th Annual Meeting of the Society for Neuroscience, San Diego, California, November 16th, 2010.

Xu, R., **Gadalla, M. M.**, Vandiver, M. S., Paul, B. D., Chakraborty, A., Sen, N., Ma, T. M., Mustafa, A. K., & Snyder, S. H. Hydrogen sulfide causes *S*-sulfhydration of PTEN and decreases its activity. Neuroscience 2010, the 40th Annual Meeting of the Society for Neuroscience, San Diego, California, November 14th, 2010.

Vandiver, M. S., Xu, R., Ko, H. S., **Gadalla, M. M.**, Dawson, V. L., Dawson, T. M., & Snyder, S. H. Hydrogen sulfide enhances parkin activity through protein *S*-sulfhydration. Neuroscience 2010, the 40th Annual Meeting of the Society for Neuroscience, San Diego, California, November 16th, 2010.

Gadalla, M. M., & Hess, G. P. Discovery of a cocaine binding site on β-amyloid peptides which play an important rôle in Alzheimer's disease. The 51st Annual Meeting of the Biophysical Society, Baltimore, Maryland, March 5th, 2007.

Gadalla, M. M., & Hess, G. P. Discovery of a new regulatory site on beta-amyloid peptides. The 10th International Conference on Alzheimer's Disease and Related Disorders (ICAD), Madrid, Spain, July 19th, 2006.

Gadalla, M. M., Lewis, R. W., & Hess, G. P. Discovery of a regulatory cocaine-binding site on β-amyloid peptides. Pharmacology Graduate Poster Party, Cornell University, Ithaca, New York, June 17th, 2006.

Gadalla, M. M., & Hess, G. P. Unraveling the mystery—aptamers, the nicotinic acetylcholine receptor, and β-amyloid peptides. Cornell Engineering Alumni Association (CEAA) Conference Poster session, Cornell University, Ithaca, New York, April 21st, 2006.

Gadalla, M. M., & Hess, G. P. Discovery of a new regulatory site on β-amyloid peptides. The 4th Annual Bioengineering Expo, Institute of Biological Engineers, Cornell University, Ithaca, New York, March 1st, 2006.

Patents:

Snyder, S. H., **Gadalla, M. M.**, Prabhakar, N. R., Stein G., & Pace, G., inventors; Johns Hopkins University, The University of Chicago, & Sova Pharmaceuticals, Inc., assignee. Methods for treatment of sleep-related breathing disorders. WO/2011/130181. April 13th, 2010.

Gadalla, M. M., & Hess, G. P., inventors; Cornell Research Foundation, Inc., assignee. Inhibition of beta-amyloid peptide aggregation. WO/2008/008884. July 12th, 2006.

Teaching Experience:

Teaching Assistant, Johns Hopkins University School of Medicine

Fall 2009	Scientific Foundations of Medicine		*Peter N. Devreotes*

Graduate Student Tutor, Johns Hopkins University School of Medicine

2008–2017 Tutored Graduate Students in the Pharmacology and Molecular Sciences Graduate Program in Molecular Biology and Genomics (ME:260.709), Cell Structure and Dynamics (ME:110.728), Organic Mechanisms in Biology (ME:330.709), and Mechanisms in Bio-Organic Chemistry (ME:330.710).

Laboratory Instructor, Cornell University

Fall 2005	ChemE 432	Unit Operations Laboratory	*Alfred M. Center*

Teaching Assistant, Cornell University

Spring 2007	ChemE 462	Chemical Process Design	*Jeffrey D. Varner*
Fall 2005	EngrI 112	Intro to Chemical Eng.	*T. Michael Duncan*
Spring 2005	ChemE 323	Fluid Mechanics	*Donald L. Koch*

Learning Initiative for Future Engineers Tutor, Cornell University

2004–2007 Tutored Engineering Undergraduate Students in Physics, Differential Equations, Fluid Mechanics, and Physical Chemistry.